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American Journal  
of Medicine



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*The American Journal of Medicine is published monthly by The American Journal of Medicine, Inc., 11 East 36th Street, New York 16, N. Y. Yearly Subscription, \$12.00 U. S. A.; \$13.00 Canada; \$16.00 Foreign, including Latin-American countries, Single Numbers \$2.00; Special and Symposium Numbers \$4.00. Second-class postage paid at New York, N. Y. and at additional mailing offices, May 1959—Volume XXVI, No. 5. Copyright © 1959, by The American Journal of Medicine, Inc. No part of the contents of this publication may be reproduced or distributed without the express written consent of the publishers.*

MANUSCRIPTS: All manuscripts should be typewritten double space and addressed to the Editorial Office of the Journal, 11 East 36th St., New York 16, N. Y. The top should be indicated on the back of each photograph. *Style for bibliography:* DOE, J. J. Treatment of hypertension. *Am. J. Med.*, 6: 72, 1948.

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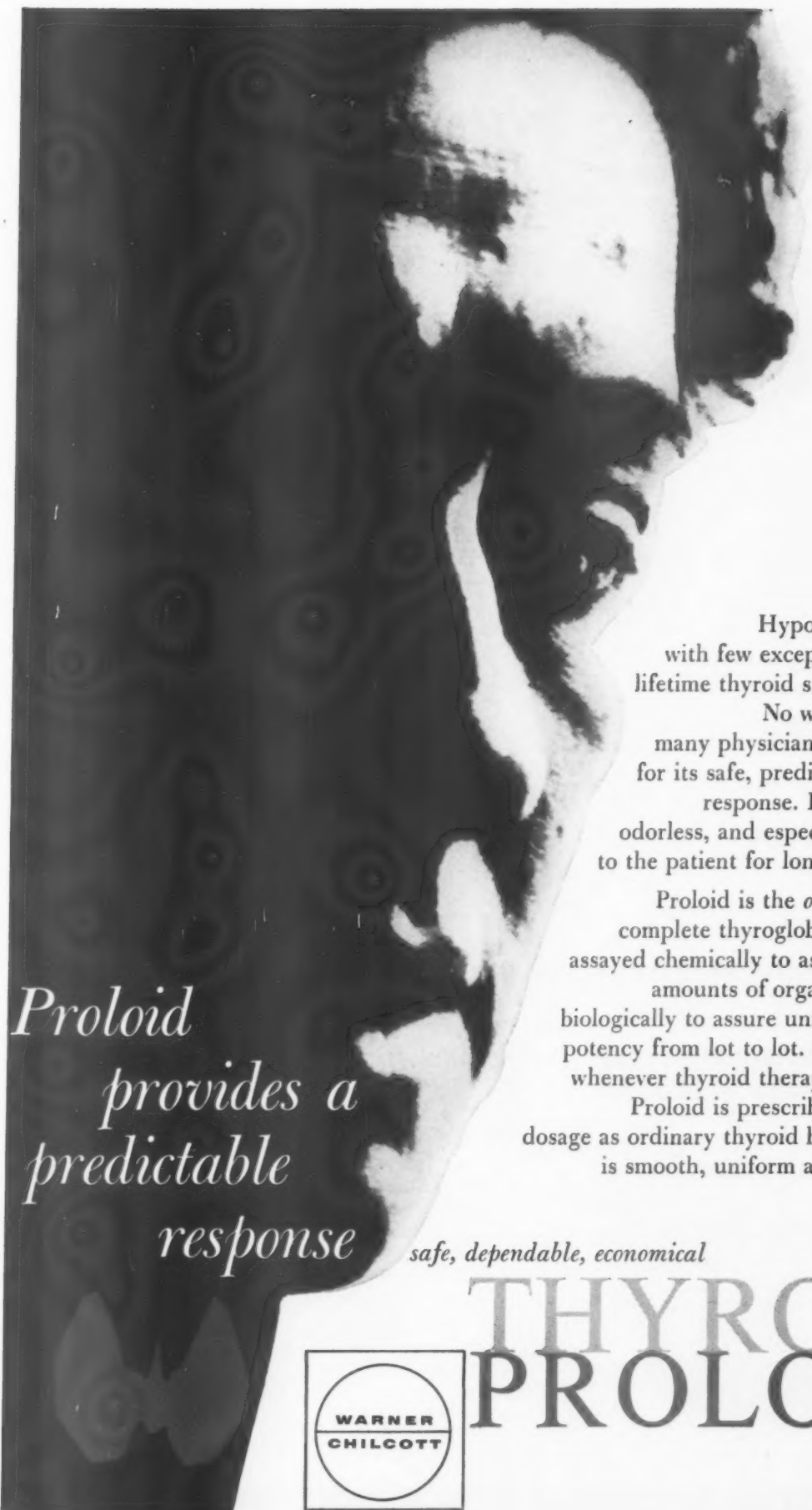
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(1) Rubin, A., and Babbott, D.: J.A.M.A. 168:498, (Oct. 4) 1958. (2) Kinsey, A. C.; Pomeroy, W. B., and Martin, C. E.: Sexual Behavior in the Human Male, Philadelphia, W. B. Saunders Company, 1948.

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# The American Journal of Medicine

Vol. XXVI MAY 1959 No. 5

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- The Concept of Functional Coarctation of Large Blood Vessels  
DALI J. PATEL, RAMON L. LANGE AND HANS H. HECHT 761

Since anatomical constriction of the aorta does not necessarily result in hemodynamic alterations, it is important in some cases to assess the functional liability of the anomaly. The present study describes a quantitative method for evaluating significant obstruction to flow by use of two arterial catheters, proximal and distal to the coarctation, and analysis of the changes in systolic and mean pressures, and of the slopes obtained. Such analysis should assist in selection of patients appropriate for surgical intervention.

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When anxiety and tension complicate **HIGH  
BLOOD  
PRESSURE**

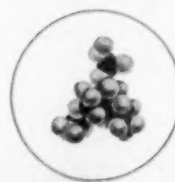


Treatment is more successful when  
mental pressure and blood pressure  
are treated together **Miltown**

Miltown in addition to ganglionic blocking therapy resulted in subjective and objective improvement in 35 of 37 patients. On the antihypertensive agent alone, only 28 patients improved.<sup>1</sup>

1. Nussbaum, H. E., Leff, W. A., Mattia, V. D., Jr. and Hillman, E.: An effective combination in the treatment of the hypertensive patient. *Am. J. M. Sc.* **234**: 150, Aug. 1957.

*Literature  
and samples  
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## *CONTENTS continued—May 1959*

VOLUME TWENTY-SIX

NUMBER FIVE

### Cortisone-Induced Polyuria Following Hypophysectomy

J. S. ROBSON AND ANNE T. LAMBIE 769

Polyuria, not uncommon in hypophysectomized subjects treated with cortisone, was found in two such patients to be related primarily to a disturbance in the thirst mechanism, and polydipsia; not, as has been suggested, to true diabetes insipidus or to any intrinsic abnormality in renal mechanisms for reabsorption of water. Cortisone seems, paradoxically, to increase the volume of water capable of being reabsorbed, while producing polyuria and polydipsia. Explanations of this paradox vary. The authors favor the view that, under conditions of osmotic diuresis, cortisone acts directly upon the renal concentrating mechanism, increasing the reabsorption of solute-free water. They invoke the Wirz countercurrent hypothesis to relate this increased reabsorption of solute-free water to the action of cortisone and other adrenocorticoid steroids in increasing the reabsorption of sodium.

### Primary Lymphosarcoma of the Stomach. A Clinical Study of Seventy-five Cases

A. I. FRIEDMAN 783

This is an account of sixty-four cases of primary small round cell lymphosarcoma of the stomach and eleven cases of reticulum cell lymphosarcoma, all histologically verified, seen over a twenty-year span, with prolonged follow-up of many of the surgically treated patients. The experience confirms the general conviction that primary lymphosarcoma of the stomach cannot be distinguished with assurance from carcinoma by any means short of tissue examination. The prognosis, after proper surgical intervention and postoperative radiotherapy, is rather more favorable than anticipated, even when lymph node involvement is demonstrated. Seven patients survived more than ten years, with no evidence of recurrence.

### *Seminar on Connective Tissue*

#### Remarks on the Present State of the Rheumatoid Arthritis Problem CHARLES RAGAN 797

It is important in any great enterprise, including investigation of the cause and cure of rheumatoid arthritis, to take a fresh, sober look now and then at the present status. Candor compels Dr. Ragan to render a rather discouraging report in the sense that while much necessary spade work has been accomplished in respect to the natural history of rheumatoid arthritis, the basic anatomy and physiology of the connective tissues, and the serological characteristics of the disorder, it is not yet clear that the data thus far accumulated are anything more than peripheral to the central issues. On the other hand, discovery comes most easily to the prepared mind, and there is no telling just where the breakthrough may occur.

### *Case Reports*

#### Parathyroid and Pancreatic Adenomas

BURTON D. COHEN, GLENN D. LUBASH AND ALBERT L. RUBIN 801

A case of unusual interest.

*Contents continued on page 7*

*new*  
*"flavor-timed"*  
*dual-action*  
*coronary vasodilator*

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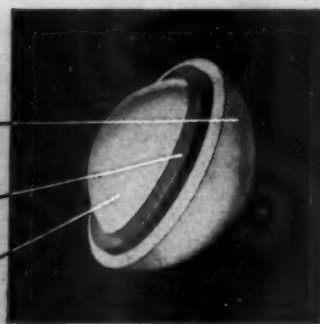
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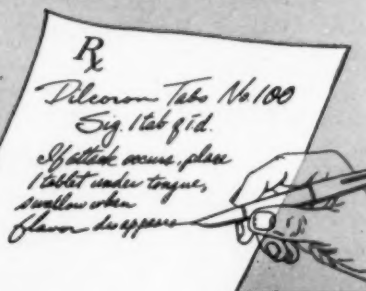
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# CONTENTS continued—May 1959

VOLUME TWENTY-SIX

NUMBER FIVE

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## The Primary Hypoventilation Syndrome THEODORE RODMAN AND HENRY P. CLOSE 808

This case is of unusual interest, representing the fifth reported patient with primary hypoventilation. As there was no demonstrable pulmonary etiology for the hypoventilation, the respiratory center in the central nervous system was implicated.

## Hyperthyroidism Associated with Renal Tubular Acidosis. Discussion of Possible Relationship EDWARD J. HUTH, ROBERT L. MAYOCK AND ROBERT M. KERR 818

Renal tubular acidosis appears to have developed in the course of hyperthyroidism in this patient, suggesting the possibility that the hypercalciuria and hypercalcemia noted in association with the hyperthyroidism might have led to renal tubular damage and a deficiency in ammonium production.

*Advertising Index on Page 119*

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**References:** 1. Crunk, G. A.; Naumann, D. E., and Casson, K.: *Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia Inc. 1958, p. 397.  
2. Newcomer, V. D.; Wright, E. T., and Sternberg, T. H.: *Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia Inc., 1955, p. 686.

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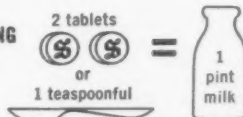
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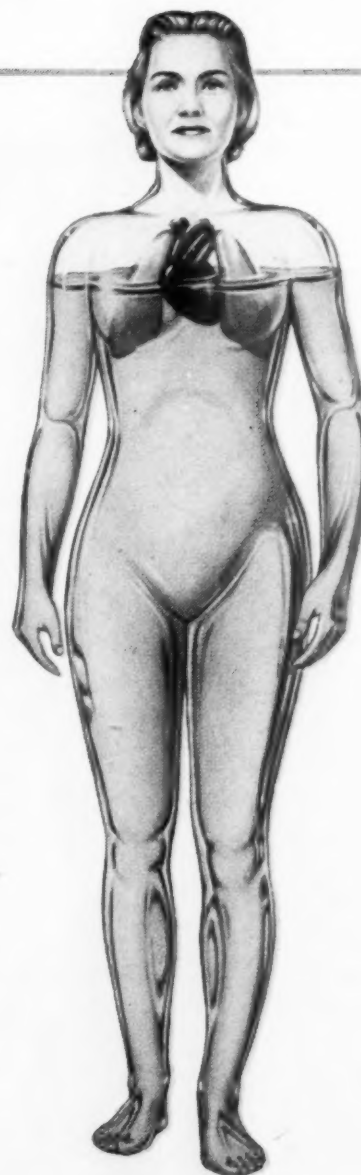
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**bibliography:** 1. Esch, A. F., Wilson, I. M. and Freis, E. D.: 3,4-Dihydrochlorothiazide: Clinical Evaluation of a New Saluretic Agent. Preliminary Report; M. Ann. District of Columbia **28**:9, (Jan.) 1959. 2. Ford, R. V.: The Clinical Pharmacology of Hydrochlorothiazide; Southern Med. J. **52**:40, (Jan.) 1959. 3. Fuchs, M., Bodi, T., Irie, S. and Moyer, J. H.: Preliminary Evaluation of Hydrochlorothiazide ('HYDRODIURIL'); M. Rec. & Ann. **51**:872, (Dec.) 1958. 4. Moyer, J. H., Fuchs, M., Irie, S. and Bodi, T.: Some Observations on the Pharmacology of Hydrochlorothiazide; Am. J. Cardiol. **3**:113, (Jan.) 1959.



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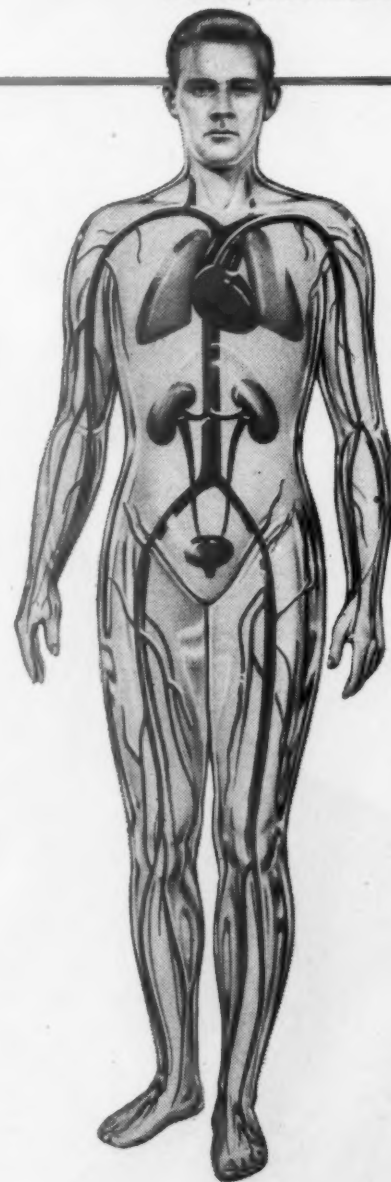
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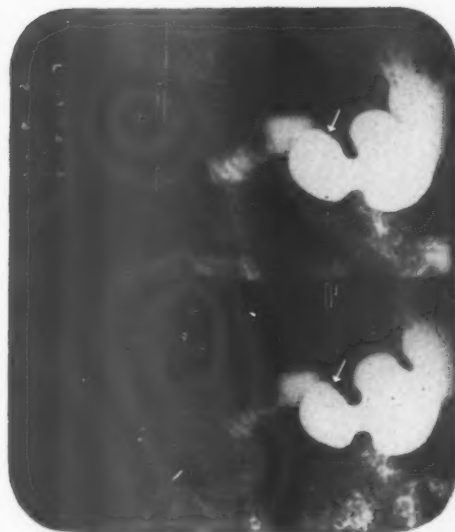


relieve the tension



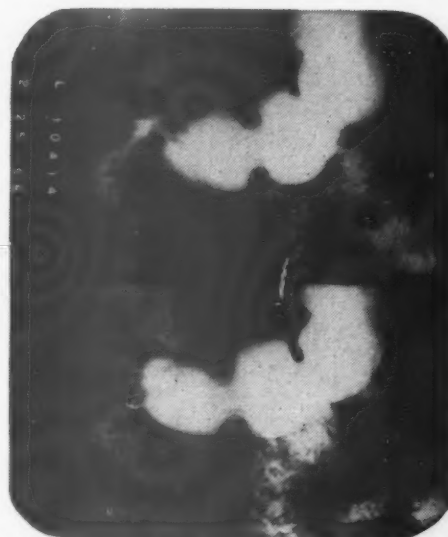


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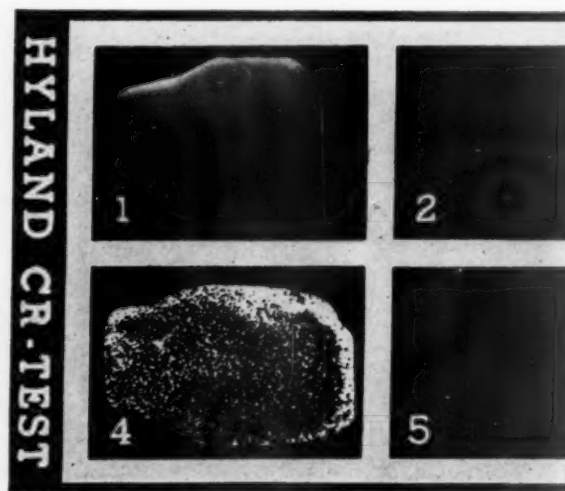
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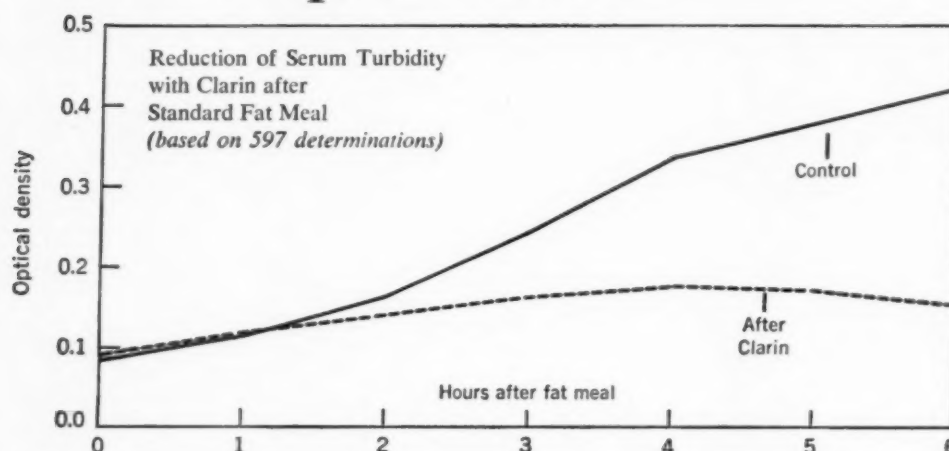
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1. Council on Drugs, J.A.M.A. 166:52 (Jan. 4) 1958.
2. Hahn, P. F.: Science 98:19 (July 2) 1943.
3. Fuller, H. L.: Angiology 9:311 (Oct.) 1958.
4. Rubio, F. A., Jr.: Personal communication.
5. Engelberg, H., et al.: Circulation 13:489 (April) 1956.

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Semoxydrine <sup>®</sup> HCl . . . . .	5 mg.
(Methamphetamine HCl)	
Pentobarbital . . . . .	20 mg.
Ascorbic Acid . . . . .	100 mg.
Thiamine Mononitrate . . . . .	0.5 mg.
Riboflavin . . . . .	1 mg.
Nicotinic Acid . . . . .	5 mg.



**OBEDRIN PROVIDES:**

- Methamphetamine for its proven anorexigenic and mood-lifting effects.
- Pentobarbital as a balancing agent, to guard against excitation.
- Vitamins B<sub>1</sub> and B<sub>2</sub> plus niacin to supplement the diet.
- Ascorbic acid to aid in the mobilization of tissue fluids.

Bristol, Tennessee • New York • Kansas City • San Francisco **THE S. E. MASSENGILL COMPANY**

*for effective timing...a flexible dosage form*

*tablets  
or capsules*



LUNCH



DINNER



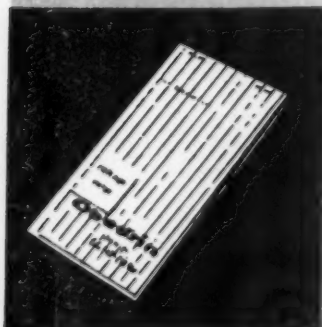
EVENING SNACK

Obedrin tablets or capsules provide a flexible dosage form which may be prescribed to depress the appetite at peak hunger periods.

The pentobarbital content assures control of excess central nervous stimulation, and the 60-10-70 Basic Plan provides for a balanced food intake with sufficient protein and roughage.

Obedrin is available in tablet and capsule form.

Currently, mailings will be forwarded only at your request. Write for 60-10-70 menus, weight charts, and samples of Obedrin



#### ADVANTAGES OF OBEDRIN

- An effective anorexigenic agent
- A flexible dosage form
- Minimal central nervous stimulation
- Vitamins to supplement the diet
- No hazards of impaction

# Obedrin<sup>®</sup>

and the 60-10-70 Basic Plan



# for nausea and vomiting **VESPRIN**

Squibb Triflupromazine

- postoperatively
- in pregnancy when vomiting is persistent
- following neurosurgical diagnostic procedures
- in infections, intra-abdominal disease, and carcinomatosis
- after nitrogen mustard therapy

- provides prompt, potent, and long-lasting control
- capable of depressing the gag reflex
- effective in cases refractory to other potent antiemetic agents
- may be given intravenously, intramuscularly and orally
- no pain or irritation on injection

#### ANTIEMETIC DOSAGE:

*Intravenous:* 8 mg. average single dose  
Dosage range 2-10 mg.

*Intramuscular:* 15 mg. average single dose  
Dosage range 5-15 mg.

If subsequent parenteral dose is needed,  
one-half the original dose will usually suffice

*Oral:* 10-20 mg. initially; then 10 mg. t.i.d.

#### SUPPLY:

*Parenteral solution* — 1 cc. ampuls (20 mg./cc.),  
1 cc. multiple dose vials (20 mg./cc.)

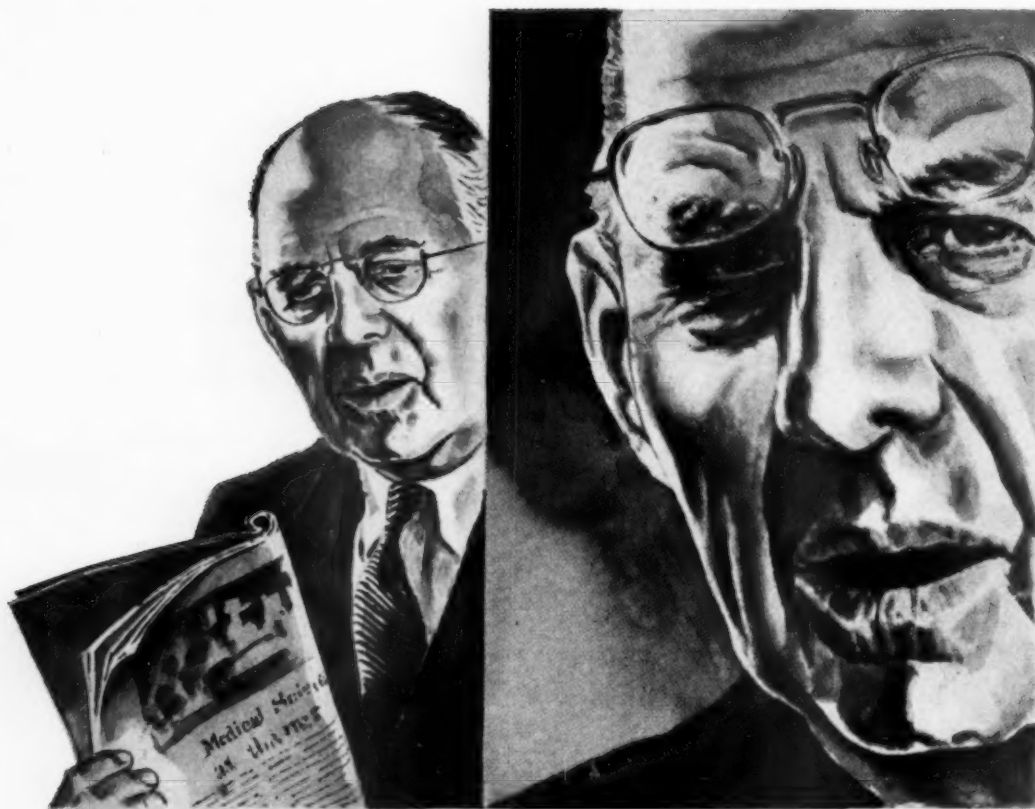
*Oral tablets* — 10 mg., 25 mg., 50 mg., in  
bottles of 50 and 500

SQUIBB



Squibb Quality — The Priceless Ingredient

"VESPRIN"® IS A SQUIBB TRADEMARK



## *"Doctors can't help shingles?"*

Physicians who have used PROTAMIDE extensively deplore such statements as unfortunate when they appear in the lay press. They have repeatedly observed in their practice quick relief of pain, even in severe cases, shortened duration of lesions, and greatly lowered incidence of postherpetic neuralgia when PROTAMIDE was started promptly. A folio of reprints is available. These papers report on zoster in the elderly—the severely painful cases—patients with extensive lesions. PROTAMIDE users know "shingles" *can* be helped.



# **PROTAMIDE®**

*Sherman Laboratories*

Detroit 11, Michigan

Available: Boxes of 10 ampuls—prescription pharmacies.

"Its relative simplicity  
makes it very acceptable  
to the patient."\*

# Delfen

ORTHO'S MOST SPERMICIDAL CONTRACEPTIVE



## THE HEART DISEASE PATIENT NEEDS RELIEF FROM EMOTIONAL STRESS



ANXIETY INTENSIFIES the physical disorder in heart disease. "The prognosis depends largely on the ability of the physician to control the anxiety factor, as well as the somatic disease."

(Friedlander, H. S.: The role of ataraxics in cardiology. *Am. J. Cardiol.* 1:395, March 1958.)

TRANQUILIZATION WITH MILTOWN enhances recovery from acute cardiac episodes and makes patients more amenable to necessary limitations of activities.

(Waldman, S. and Perner, L.: Management of anxiety associated with heart disease. *Am. Pract. & Digest Treat.* 8:1075, July 1957.)

# Miltown<sup>®</sup>


meprobamate (Wallace)

Available in 400 mg. scored and 200 mg. sugar-coated tablets. Also available as MEPROSPAN\* (200 mg. meprobamate *continuous release* capsules). In combination with a nitrate, for angina pectoris: MILTRATE\*—(Miltown 200 mg. + PETN 10 mg.).

\*TRADE-MARK

CM-7726

Miltown causes no adverse effects on heart rate, blood pressure, respiration or other autonomic functions.

 WALLACE LABORATORIES, New Brunswick, N. J.



Restore summer warmth  
to cold, aching extremities

# ILIDAR

- Increases peripheral circulation
- Relieves vasospasm

"Highly effective"\* in vasospastic disorders, Ilidar promptly alleviates symptoms of cold, numb, aching extremities, with virtual freedom from unwanted side reactions. Ilidar, unlike most vasodilators, is exceptionally well tolerated: "one of the most pleasant drugs in its class to use."\*

Prescribe Ilidar in peripheral vasospastic disorders to relieve aching, burning, coldness, night cramps and numbness of the extremities.

\*H. D. Green and H. H. DuBose, Circulation, 10:374, 1954.

**SUPPLIED:** 25-mg tablets, in bottles of 100 and 500 tablets.

**ILIDAR®**—brand of azapetine



**ROCHE** LABORATORIES

Division of Hoffmann-La Roche Inc.

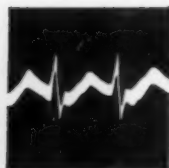
Nutley 10, New Jersey



*"nutrition... present as a modifying or complicating factor in nearly every illness or disease state"<sup>1</sup>*

# the rationale for

## *in* cardiac disease



"B vitamins should be an integral part of the treatment prescribed for any patient with cardiac disease. . . . As a consequence of special low salt diets and diuretics prescribed to release the water held in the body fluids by an excess of sodium, the B vitamins are 'washed out' of the body with the salt, and the difficulties of the disease are compounded."<sup>2</sup>

## *in* infectious disease



"There are ample, critical, statistically significant studies to indicate that good nutrition is important for optimal resistance to infection, for a superior tissue capability to cope with disease and injury, and for maximum antibody formation."<sup>3</sup>

"Fever also increases vitamin requirements. This is especially true of the B-complex and C vitamins. Liquid and soft diets, which are commonly prescribed early in disease, are inadequate in these vitamins. It is advisable to give supplementary vitamin capsules during the actual illness and convalescence."<sup>6</sup>

### *Each Theragran supplies:*

Vitamin A . . . . .	25,000 U.S.P. units
Vitamin D . . . . .	1,000 U.S.P. units
Thiamine Mononitrate . . . . .	10 mg.
Riboflavin . . . . .	10 mg.
Niacinamide . . . . .	100 mg.
Ascorbic Acid . . . . .	200 mg.
Pyridoxine Hydrochloride . . . . .	5 mg.
Calcium Pantothenate . . . . .	20 mg.
Vitamin B <sub>12</sub> Activity Concentrate . . . . .	5 mcg.

*Dosage:* 1 or more daily as indicated.

*Supply:* Family Packs of 180. Bottles of 30, 60, 100 and 1,000.

### *.....* **THERAGRAN with Minerals** **available as THERAGRAN-M**

(SQUIBB VITAMIN-MINERALS FOR THERAPY)

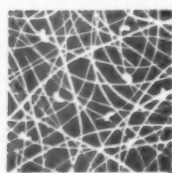
*bottles of 30, 60, 100 and 1,000*  
*capsule-shaped tablets and*  
*Family Packs of 180.*  
*.....*

*Also available:* Theragran Liquid, bottles of 4 ounces; Theragran Junior, bottles of 30 and 100.

*References:* 1. Youmans, J. B.: *Am. J. Med.* 25:659, Nov. 1958. 2. Gertler, M. M.: Paper presented at Conference on Metabolic Factors in Cardiac Contractility, N. Y. Acad. Sciences, New York City, N. Y., March 18-19, 1958. 3. Fernandy-Herlihy, L.: *Lahey Clinic Bull.* 11:12, July-Sept. 1958. 4. Spies, T. D.: *J.A.M.A.* 167:675, June 7, 1958. 5. Halpern, S. L.: *Ann. N. Y. Acad. Sci.* 3:147, Oct. 28, 1955. 6. Pollack, H., and Halpern, S. L.: *Therapeutic Nutrition*, National Academy of Sciences and National Research Council, Washington, D. C., 1952, p. 54. 7. Kountz, W. B.: *Mod. Med.* 25:102, Aug. 1, 1957. 8. Sebrell, W. H.: *Am. J. Med.* 25:673, Nov. 1958.

# the use of vitamins

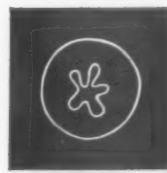
## *in rheumatoid arthritis*



"It is our practice to prescribe a multiple vitamin preparation to patients with rheumatoid arthritis [collagen disease] simply to insure nutritional adequacy..."<sup>3</sup>

"Many rheumatologists now look for nutritive failure among the patients who have arthritis and other debilitating diseases."<sup>4</sup>

## *in degenerative disease*



"Most degenerative disease changes are believed to be related to disturbed nutrition. ... Even though blood levels may be adequate [for vitamin A, vitamin D, thiamine, ascorbic acid, and riboflavin]...many individuals will improve with supplementary administration."<sup>7</sup>

"In chronic diseases...in which there is a loss of appetite, difficulty in eating or abnormal metabolic demand, symptoms of B vitamin deficiencies also have been found frequently and should always be looked for in their management."<sup>8</sup>

*for the next patient you see who needs nutritional support*

# Theragran

SQUIBB VITAMINS FOR THERAPY

**SQUIBB**



*Squibb Quality — the Priceless Ingredient*

"Theragran"® is a Squibb trademark.

**NOW**

*...a new way  
to relieve pain  
and stiffness  
in muscles  
and joints*

INDICATED IN:

MUSCLE STIFFNESS

LUMBOSACRAL STRAIN

SACROILIAC STRAIN

WHIPLASH INJURY

BURSITIS

SPRAINS

TENOSYNOVITIS

FIBROSITIS

FIBROMYOSITIS

LOW BACK PAIN

DISC SYNDROME

SPRAINED BACK

"TIGHT NECK"

TRAUMATIC STRAINS  
AND BRUISES

POSTOPERATIVE  
MYALGIA





- Exhibits unusual analgesic properties, different from those of any other drug
- Specific and superior in relief of SOMATIC pain
- Modifies central perception of pain without abolishing natural defense reflexes
- Relaxes abnormal tension of skeletal muscle

# SOMA<sup>TM</sup>

N-isopropyl-2-methyl-2-propyl-1, 3-propanediol dicarbamate

- More specific than salicylates
- Less drastic than steroids
- More effective than muscle relaxants

**SOMA** has an unique analgesic action. It apparently modifies central pain perception without abolishing peripheral pain reflexes. **SOMA** is particularly effective in relieving joint pain. Patients say that they feel better and sleep better with **SOMA** than with any previously used analgesic, sedative or relaxant drug.

**SOMA** also relaxes muscle hypertonia, with its stresses on related joints, ligaments and skeletal structures.

**ACTS FAST.** Pain-relieving and relaxant effects start in 30 minutes and last 6 hours.

**NOTABLY SAFE.** Toxicity of **SOMA** is extremely low. No effects on liver, endocrine system, blood pressure, blood picture or urine have been reported. Some patients may become sleepy on high dosage.

**EASY TO USE.** Usual adult dose is one 350 mg. tablet 3 times daily and at bedtime.

**SUPPLIED:** Bottles of 50 white sugar-coated 350 mg. tablets.

*Literature and samples on request.*



WALLACE LABORATORIES, NEW BRUNSWICK, N. J.

# With Singoserp this patient's blood pressure was controlled for the first time without side effects

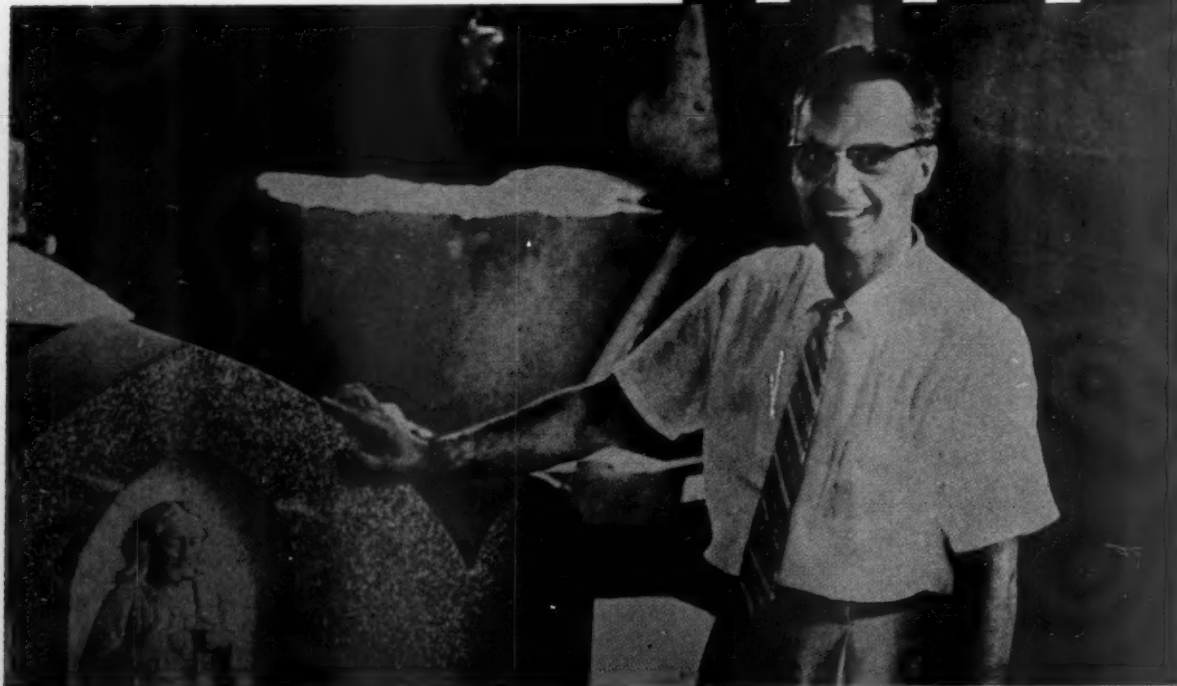
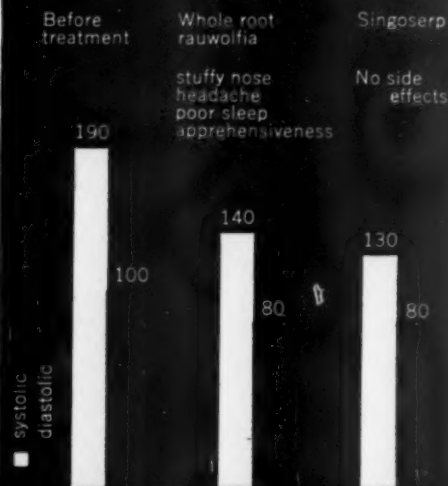
FROM THE FILES OF A PHILADELPHIA CARDIOLOGIST.  
PHOTOS USED WITH PERMISSION OF THE PATIENT.

Tombstone salesman had known hypertension for 16 years; rejected by U.S. Army because of high blood pressure. Whole root rauwolfia lowered pressure satisfactorily, but patient could not tolerate side effects.

Singoserp in a dosage of 0.5 mg. daily lowered his blood pressure to 130/80, produced no side effects. Patient feels well, works well, speaks of marked improvement in outlook and function.



History of this patient in chart form:



## Clinical findings in 900 patients show the selective antihypertensive action of Singoserp

IN 735 PATIENTS, BLOOD PRESSURE FELL AN AVERAGE OF 30.7 mm. Hg:

- more than half of these patients suffered from moderate to severe hypertension
- more than half of the cases involved hypertension of at least 6 years' standing, with many histories of up to 20 years' duration

THE SIDE-EFFECTS PROBLEM WAS MINIMIZED IN MOST PATIENTS:

Chart shows gratifyingly low incidence of side effects in 233 patients given Singoserp with no other antihypertensive medication

Side Effect	Number	Per Cent
Lethargy	7	2.9
Headache	6	2.5
Gastrointestinal upset	3	1.2
Vertigo	2	0.8
Nasal congestion	1	0.4

### DOSAGE:

*In new patients:* Average initial dose, 1 to 2 tablets (1 to 2 mg.) daily. Some patients may require and will tolerate 3 or more tablets daily. Maintenance dose will range from ½ to 3 tablets (0.5 to 3 mg.) daily.

*In patients taking other antihypertensive medication:* Add 1 to 2 Singoserp tablets (1 to 2 mg.) daily. Dosage of other agents should be revised downward to a level affording maximal control of blood pressure and minimal side effects.

# Singoserp®

(syrosingopine CIBA)

C I B A  
SUMMIT N. J.

a major  
improvement  
in rauwolfia

a major  
advance in  
antihypertensive  
therapy



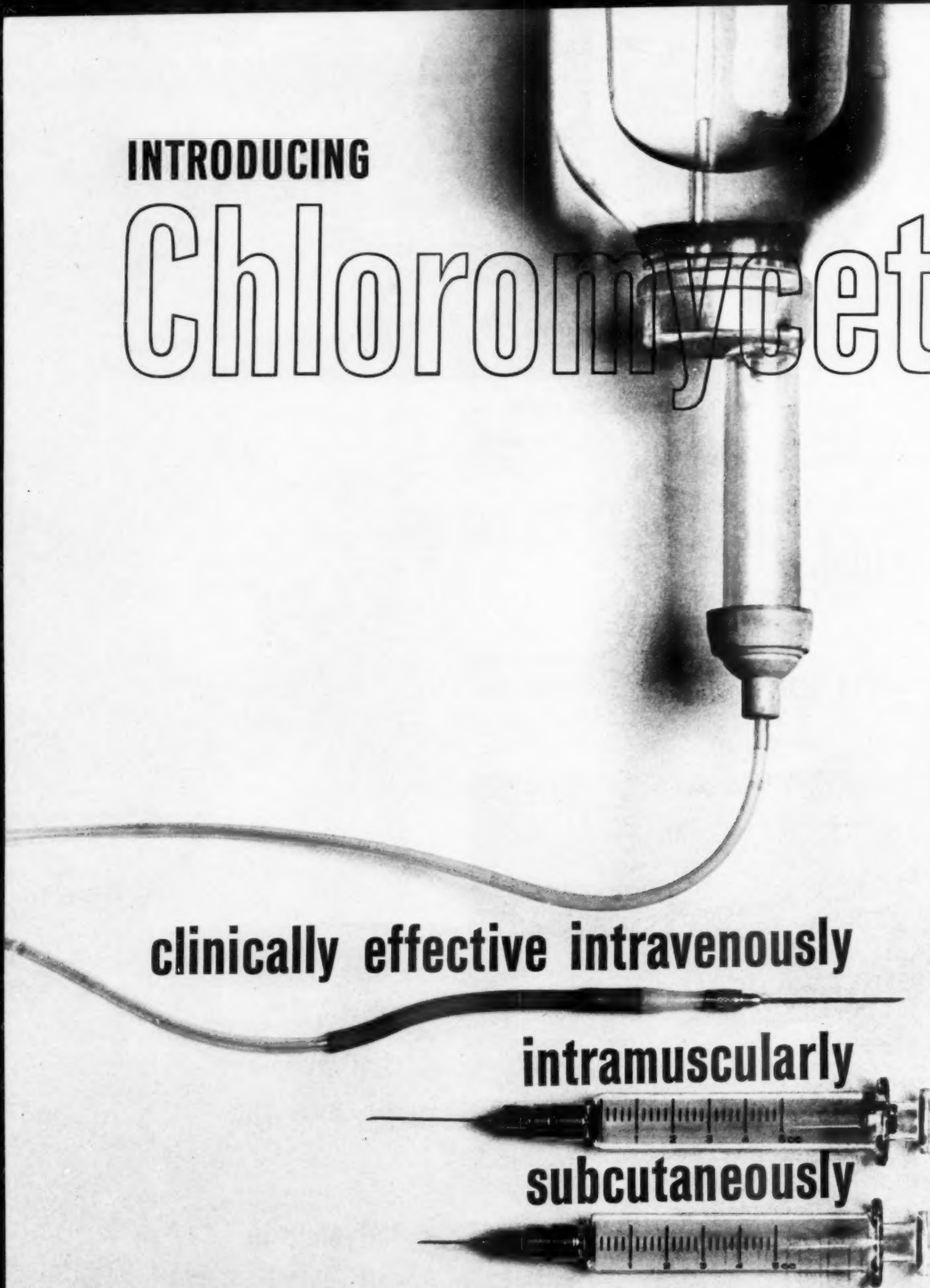
**INTRODUCING**

# Chloromycetin<sup>®</sup>

**clinically effective intravenously**

**intramuscularly**

**subcutaneously**





# Succinate

"...a distinct advance in parenteral chloramphenicol therapy,"<sup>1</sup> CHLOROMYCETIN SUCCINATE is a soluble ester of CHLOROMYCETIN that can be administered intramuscularly, intravenously, or subcutaneously. CHLOROMYCETIN SUCCINATE is rapidly hydrolyzed by body esterases and produces effective blood and tissue concentrations of CHLOROMYCETIN within a short time.<sup>2</sup> Tissue reaction at the site of injection is minimal,<sup>2</sup> permitting continuous daily dosage, even in pediatric patients.<sup>1</sup>

## WIDE-SPECTRUM ANTIMICROBIAL EFFECTIVENESS

CHLOROMYCETIN SUCCINATE, providing broad-spectrum antimicrobial effectiveness, may be used whenever CHLOROMYCETIN is indicated. It has produced effective response in respiratory, gastrointestinal, and rickettsial infections.<sup>1,3,4</sup> Because of the rapid, effective blood levels of CHLOROMYCETIN provided, it is especially useful in *Hemophilus influenzae*

meningitis, in certain septicemias, typhoid fever, and other *Salmonella* infections.<sup>1,4</sup>

## WELL TOLERATED

CHLOROMYCETIN SUCCINATE is well tolerated, even by small children. Signs of irritation at injection sites have been few.<sup>1-4</sup>

**DOSAGE AND ADMINISTRATION** — *Adults*: 1 Gm. every six to eight hours. *Children*: 100 mg. per Kg. of body weight per day in divided doses at six-to eight-hour intervals. The total dose in children should not exceed the adult dose of 1 Gm. given at any single injection, with exception of treatment of *Hemophilus influenzae* meningitis in which higher doses are employed.

In all cases, severity of infection and clinical response to therapy should be the guiding factors determining the proper dosage schedule. Premature and full-term newborn infants require special dosage supervision. For details see literature.

## TYPICAL CLINICAL EXPERIENCE WITH CHLOROMYCETIN SUCCINATE

Type of infection	Number of Patients	RESULTS		
		Excellent to Good	Fair	Poor
Respiratory <sup>1,3*</sup>	32	32		
<i>Shigella</i> dysentery <sup>1</sup>	14	14		
Enteritis <sup>1</sup>	10	6	2	2
Bacteremia <sup>1,4</sup>	5	5		
Meningitis <sup>1,4</sup>	4	3		1**
Rocky Mountain spotted fever <sup>1,4</sup>	2	2		
Ear abscess with cellulitis <sup>3</sup>	1	1		
Lung abscess <sup>3</sup>	1			1
Typhoid fever <sup>4</sup>	1	1		
<b>TOTALS</b>	<b>70</b>	<b>64</b>	<b>2</b>	<b>4</b>

\*Includes 15 patients who were administered CHLOROMYCETIN SUCCINATE by nebulization under intermittent positive pressure breathing.

\*\*Patient was hydrocephalic at birth; cerebrospinal fluid was sterile at time of death.

**SUPPLY** — CHLOROMYCETIN SUCCINATE (chloramphenicol sodium succinate, Parke-Davis) is supplied in Steri-Vials,<sup>®</sup> each containing the equivalent of 1 Gm. chloramphenicol; packages of 10.

CHLOROMYCETIN is a potent therapeutic agent and, because certain blood dyscrasias have been associated with its administration, it should not be used indiscriminately, or for minor infections. Furthermore, as with certain other drugs, adequate blood studies should be made when the patient requires prolonged or intermittent therapy.

**REFERENCES** — (1) Ross, S.; Puig, J. R., & Zaremba, E. A., in Welch, H., & Marti-Ibañez, E.: *Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 803. (2) Glazko, A. J., et al.: *ibid.*, p. 792. (3) Payne, H. M., & Hackney, R. L., Jr., in Welch, H., & Marti-Ibañez, E.: *Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 821. (4) McCrumb, F. R., Jr.; Snyder, M. J., & Hicken, W. J.: *ibid.*, p. 837.

PARKE, DAVIS & COMPANY • DETROIT 32, MICHIGAN

70959



# KENA

for all your  
patients  
starting on  
corticoids

Kenacort safely starts your patients off right — with all the benefits of systemic corticosteroid therapy and few side effects to worry about. Increased antiallergic, antirheumatic or anti-inflammatory activity is provided on a low dosage schedule.<sup>1-3</sup> Clinical improvement is accomplished without water or salt retention,<sup>1-4</sup> or adverse effect on blood pressure.<sup>1-3,5</sup> A low sodium diet is not necessary.<sup>4,5</sup> Gastrointestinal disturbances are negligible<sup>2,4,5</sup> with less chance of peptic ulcer,<sup>4</sup> and there is no psychic stimulation to distort the clinical response.<sup>1-3</sup> This makes Kenacort particularly valuable in treating your "problem patients" — such as the obese or hypertensive and the emotionally disturbed.

#### REFERENCES:

1. Freyberg, R.H.; Bernsten, C.A., Jr., and Hellman, L.: Arth. & Rheum. 1:215 (June) 1958.
2. Sherwood, H., and Cooke, R.A.: J. Allergy 28:97 (March) 1957.
3. Shelley, W.B.; Harun, J.S., and Pillsbury, D.M.: J.A.M.A. 167:959 (June 21) 1958.
4. Dubois, E.L.: California Med. 89:195 (Sept.) 1958.
5. Hartung, E.F.: J.A.M.A. 167:973 (June 21) 1958.

\*KENACORT® IS A SQUIBB TRADEMARK.

SQUIBB



# CORT

Squibb Triamcinolone

for all  
your allergic  
patients  
requiring  
corticoids

Kenacort, in treating your allergic patients, has proved effective where other steroids have failed. Its potent antiallergic and anti-inflammatory properties provide rapid clinical improvement on a low dosage schedule<sup>1-3</sup> with few side effects to worry about.<sup>1-5</sup>

(Kenacort is particularly valuable for your allergic patients with hypertension, cardiac disease, obesity and those prone to psychic disturbances.) In asthma, Kenacort therapy improves ventilation and increases vital capacity.<sup>2</sup>

Dyspnea and bronchospasm are usually relieved within 48 hours, and sibilant râles often disappear.

Because of its relative freedom from untoward reactions, Kenacort provides corticosteroid benefits to many patients who until now have been difficult to control.

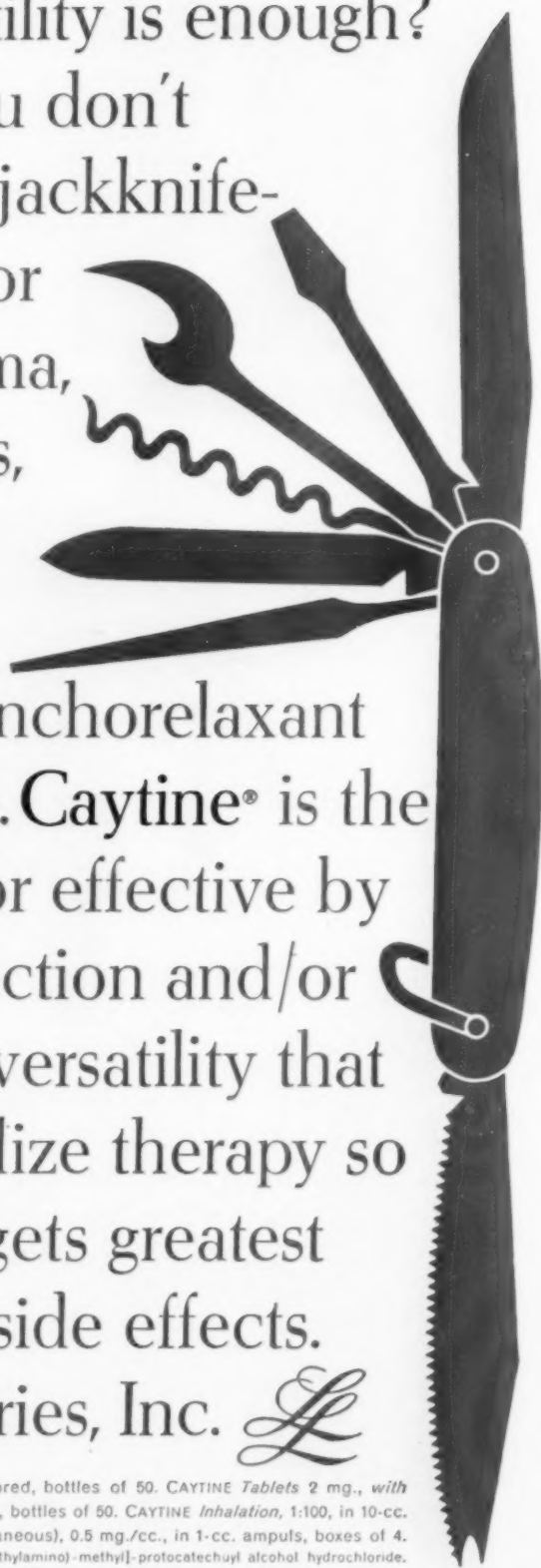
Kenacort, too, is indicated in the treatment of arthritis and dermatoses.

**SUPPLIED:**

Scored tablets of 1 mg. — Bottles of 50  
Scored tablets of 2 mg. — Bottles of 50  
Scored tablets of 4 mg. — Bottles of 30 and 100



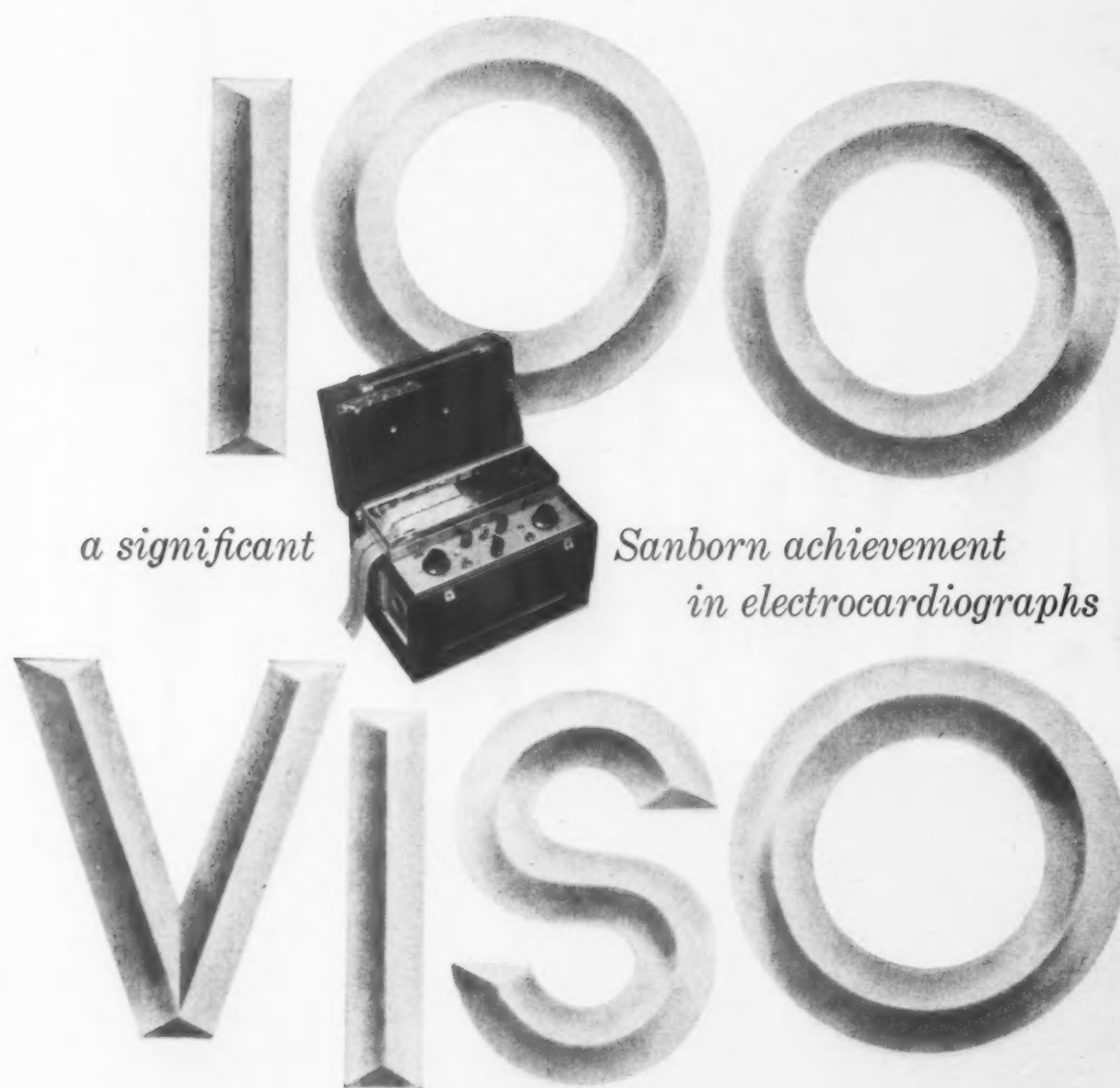
How much versatility is enough?  
That depends. You don't  
ordinarily need a jackknife-  
of-*all*-trades. But for  
asthma, emphysema,  
chronic bronchitis,  
bronchiectasis,  
you do need the  
most versatile bronchorelaxant  
you can prescribe. Caytine® is the  
first bronchodilator effective by  
mouth and/or injection and/or  
inhalation. Here's versatility that  
lets you individualize therapy so  
that each patient gets greatest  
relief with fewest side effects.  
Lakeside Laboratories, Inc. *L*



CAYTINE—3 effective forms: CAYTINE Tablets Plain, 2 mg., scored, bottles of 50. CAYTINE Tablets 2 mg., with Pentobarbital, 32 mg. (warning: may be habit forming), scored, bottles of 50. CAYTINE Inhalation, 1:100, in 10-cc. bottles with dropper. CAYTINE Injection (intramuscular, subcutaneous), 0.5 mg./cc., in 1-cc. ampuls, boxes of 4. CAYTINE is the only brand of the  $\alpha$ [( $\alpha$ -methyl-3,4-methylenedioxyphenethylamino)-methyl]-protocatechuyl alcohol hydrochloride.

40559





*a significant*

*Sanborn achievement  
in electrocardiographs*

*the completely new, 2-speed*

**SANBORN Model 100 VISO-CARDIETTE**

Here is an electrocardiograph in which *no detail* has been overlooked to give you diagnostically *accurate* information... the greatest possible operating *convenience*... and modern, *functional* attractiveness. With thirty-five years of experience, this is the finest electrocardiograph Sanborn Company has ever produced. Priced at eight hundred fifty dollars, delivered continental U. S. A.

**SANBORN COMPANY**  
MEDICAL DIVISION • 175 Wyman St., Waltham 54, Mass.

New for the metabolic treatment of

# gout

# ANTURAN<sup>TM</sup>

(sulfipyrazone GEIGY)

## High Potency Uricosuric Agent

By significantly increasing renal excretion of urate and thus lowering plasma uric acid, the new highly potent uricosuric agent ANTURAN strikes directly at the basic metabolic defect in gout.

Exceptionally high potency...4 to 6 times that of probenecid...is the outstanding characteristic of ANTURAN. The effectiveness of ANTURAN is retained indefinitely and tolerance to it is good.

## Clinically, ANTURAN:

- Prevents formation of new tophi
- Causes gradual absorption of old tophi
- Relieves chronic pain
- Restores joint mobility

ANTURAN is not designed for the treatment of acute attacks for which BUTAZOLIDIN<sup>®</sup> is recommended. Detailed Information On Request

YU, T. F.; Burns, J. J., and Gutman, A. B. Arth. & Rheumat. 1:532, 1958.

ANTURAN<sup>®</sup> (sulfipyrazone GEIGY). Scored tablets of 100 mg. in bottles of 100.

BUTAZOLIDIN<sup>®</sup> (phenylbutazone GEIGY)

Geigy  
Ardsley, New York  
98452



## Combined Orinase<sup>\*</sup>-insulin therapy enables you to "stabilize" a surprising percentage of "brittle" diabetics

The primary indication for Orinase remains in the stable, maturity-onset diabetic in whom Orinase usually can fully replace insulin therapy. But now a further indication has developed from the cumulative data of the past several years: many labile diabetics, who cannot be managed on Orinase alone, can benefit from the *addition* of Orinase to their insulin regimen.

### A major benefit—stabilization

In the labile diabetic who successfully responds to joint insulin-Orinase management, the "peaks and valleys" of erratic blood sugar levels are rarely observed. The addition of Orinase greatly reduces sudden and unexpected changes...tends to "stabilize" even the "brittle" diabetic.

### A major benefit—lessened insulin needs

The Orinase-stabilized labile diabetic generally requires less insulin than before the inclusion of Orinase in his regimen. This lessening of insulin dosage is particularly advantageous in the patient who is insulin-dependent, but who reacts unfavorably — whether by lipodystrophy or otherwise — to insulin.

### The derived benefits—less hypoglycemia, less anxiety, greater well-being

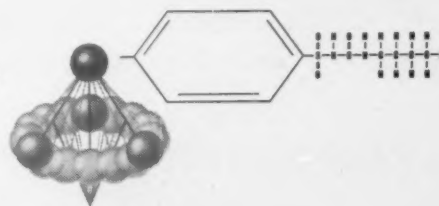
With stabilization, the hazards of shock or coma are diminished. Like the diabetic who is responsive to Orinase alone, the labile diabetic on combined therapy need no longer walk a slender tightrope between hypo- and hyperglycemia. The patient's fears are greatly lessened...often to be replaced by the healthier outlook characteristic of *euglycemic* Orinase management.

\*TRADEMARK, REG. U. S. PAT. OFF.—TOLBUTAMIDE, UPJOHN

**Upjohn**

The Upjohn Company  
Kalamazoo, Michigan

AN EXCLUSIVE  
METHYL "GOVERNOR"  
PREVENTS  
HYPOGLYCEMIA...  
MAKES ORINASE  
A TRUE  
EUGLYCEMIC AGENT



Whether the response in  
**acute skeletal  
muscle spasm**

is *"marked"*<sup>1</sup>

*"pronounced"*<sup>2</sup>

*"excellent"*<sup>3</sup>

*"significant"*<sup>4</sup>

or *"gratifying"*<sup>5</sup>—

it all adds up to

**94.4% beneficial  
results with**

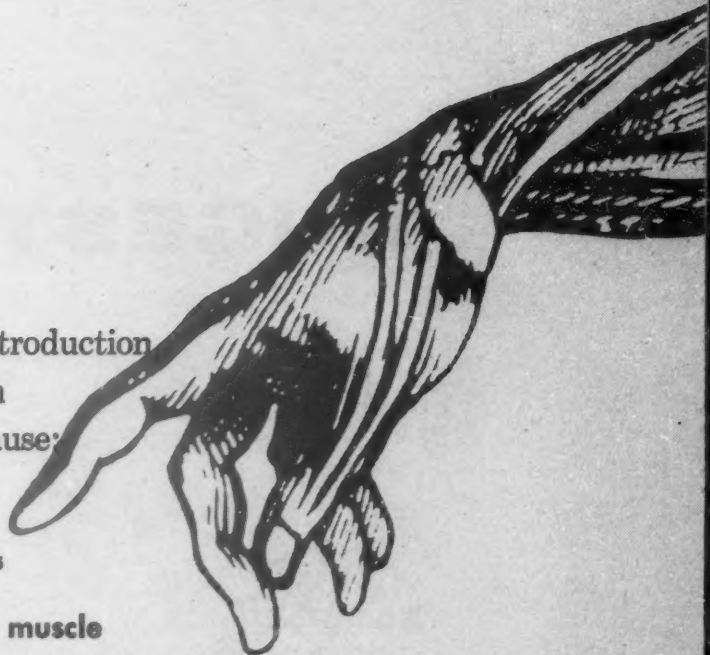
**RO**





In the comparatively short period since its introduction ROBAXIN has become the leader in prescription preference for skeletal muscle relaxation, because:

- It is highly potent—and long acting.<sup>1,2</sup>
- It is relatively free of adverse side effects.<sup>1,2,4,5</sup>
- In ordinary dosage, it does not reduce normal muscle strength or reflex activity.<sup>1</sup>



ROBAXIN's outstanding effectiveness is authenticated by the results of five recent clinical studies in which it was administered to 198 patients.<sup>1,2,3,4,5</sup> Good results were reported in 80.3% of the patients and moderate results in 14.1%—or an over-all beneficial effect in 94.4%. Conditions treated included spasm secondary to trauma, ligamentous strains, herniated disc, torticollis, whiplash injury, contusions, fractures, fibromyositis, acute myalgic disorders, and skeletal muscle spasms afflicting industrial workers.

**Supply:** ROBAXIN Tablets, 0.5 Gm. (white, scored) in bottles of 50.

**References:**

1. Carpenter, E. B.: Southern M. J. 51:627, 1958.
2. Forsyth, H. F.: J.A.M.A. 167:163, 1958.
3. O'Doherty, D. S., and Shields, C. D.: J.A.M.A. 167:160, 1958.
4. Park, H. W.: J.A.M.A. 167:168, 1958.
5. Plumb, C. S.: Journal-Lancet 78:531, 1958.



# baxin<sup>®</sup>

Methocarbamol Robins, U.S. Pat. No. 2770649

**A. H. ROBINS CO., INC., RICHMOND 20, VIRGINIA**

*Ethical Pharmaceuticals of Merit since 1878*

# NUGESTORAL®

in the treatment of  
habitual abortion

NUGESTORAL will help you bring the abortion-prone patient to term by supplying five agents known to contribute to fetal salvage. It creates an optimal environment for the maintenance of pregnancy.

## CLINICAL REPORT

Fitzgerald\* has reported a fetal salvage rate of 50% in a group of habitual aborters with the addition of NUGESTORAL to his standard regimen for abortion-prone patients.

\*Fitzgerald, W.: Clinical Medicine, 5:1037, 1958.

**Formula:** A daily dose of three NUGESTORAL tablets provides 45 mg. Progestoral® (Ethisterone); 525 mg. Ascorbic Acid; 525 mg. Hesperidin; 6 mg. Sodium Menadiol Diphosphate (Vit. K Analogue); 10.5 mg. dl, Alpha-Tocopherol Acetate (Vit. E).

**Issued:** For greater patient economy NUGESTORAL is now available in boxes of 100 gold foil wrapped tablets as well as in boxes of 30.



ORANGE, NEW JERSEY





To the relief of musculoskeletal pain,  
 new **MEDAPRIN**\*  
 adds restoration of function

Analgesics offer temporary relief of musculoskeletal pain, but they merely *mask* pain rather than getting at its *cause*. New Medaprin, in addition to bringing about prompt subjective improvement, promotes the *restoration of normal function* by suppressing the inflammation that *causes* the pain.

Medaprin, Upjohn's new analgesic-steroid combination, contains aspirin plus Medrol,\*\* the corticosteroid with the *best therapeutic ratio in the steroid field*.† Instead of suffering recurrent discomfort because of the "wearing off" of analgesics, the patient on Medaprin experiences a smooth, *extended* relief and more normal mobility.

**Indications:** Medaprin is indicated in mild-to-moderate rheumatic and musculoskeletal condi-

tions, including rheumatoid arthritis, deltoid bursitis, low back pain, neuralgia, synovitis, fibromyositis, osteoarthritis, low back sprain, traumatic wrist, sciatica, and "tennis elbow."

**Dosage:** The recommended dosage is 1 tablet q.i.d. The usual cautions and contraindications of corticotherapy should be observed.

**Supplied:** In bottles of 100 and 500.

**Formula:** Each Medaprin tablet contains


- 300 mg. acetylsalicylic acid, for prompt relief of pain
- 1 mg. Medrol, to suppress the causative inflammation
- 200 mg. calcium carbonate, as buffer

\* TRADEMARK \*\* TRADEMARK, REG. U. S. PAT. OFF. — METHYLPREDNISOLONE, UPJOHN  
 † RATIO OF DESIRED EFFECTS TO UNDESIRE EFFECTS

The Upjohn Company, Kalamazoo, Michigan

**Upjohn**





*Can antacid therapy be  
made more effective?*

ANNOUNCING

THE MOST SIGNIFICANT IMPROVEMENT IN  
ANTACID THERAPY SINCE THE INTRODUCTION  
OF ALUMINUM HYDROXIDE IN 1929

**NEW**

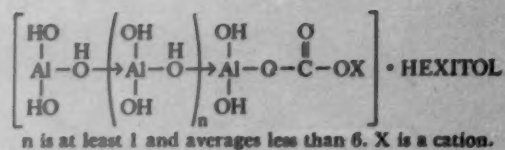
**Creamalin<sup>®</sup>** ANTACID  
TABLETS

Each Creamalin Antacid Tablet contains 320 mg. specially processed, highly reactive, short polymer dried aluminum hydroxide gel, stabilized with hexitol, with 75 mg. magnesium hydroxide.

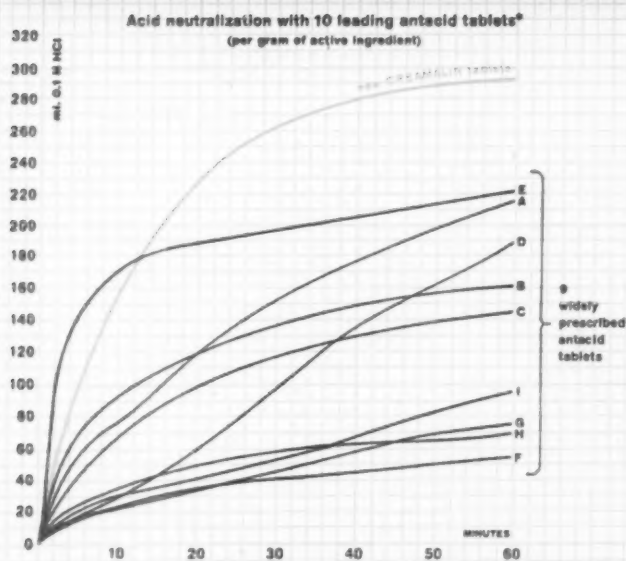
- 1. Neutralizes acid faster (quicker relief)*
- 2. Neutralizes more acid (greater relief)*
- 3. Neutralizes acid longer (more lasting relief)*
- 4. No constipation • No acid rebound*
- 5. More pleasant to take*



a new high in effectiveness  
and palatability

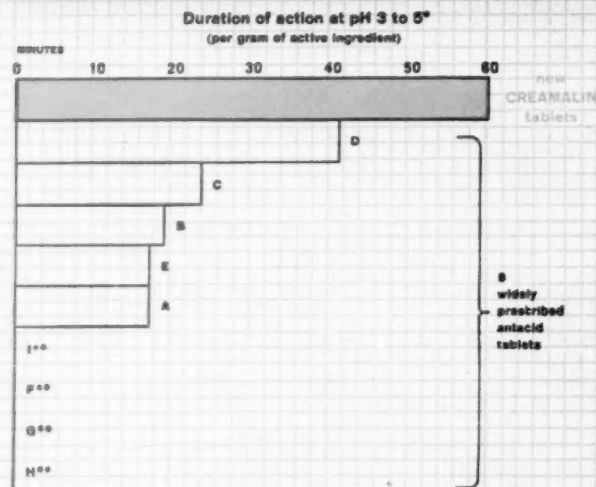


CREAMALIN neutralizes more acid faster  
Quicker Relief • Greater Relief



Tablets were powdered and suspended in distilled water in a constant temperature container (37° C) equipped with mechanical stirrer and pH electrodes. Hydrochloric acid was added as needed to maintain pH at 3.5. Volume of acid required was recorded at frequent intervals for one hour.

CREAMALIN neutralizes more acid longer  
More Lasting Relief



\*Hinkel, E. T., Jr., Fisher, M. P. and Tainter, M. L.: A new highly reactive aluminum hydroxide complex for gastric hyperacidity. To be published.  
\*\*pH stayed below 3.

Do antacids have to taste  
like chalk?



No chalky taste. New CREAMALIN tablets are not chalky, gritty, rough or dry. They are highly palatable, soft, smooth, easy to chew, mint flavored.

- NO ACID REBOUND • NO CONSTIPATION
- NO SYSTEMIC EFFECT

**Adult Dosage:** Gastric hyperacidity: 2 to 4 tablets as necessary. Peptic ulcer or gastritis: 2 to 4 tablets every two to four hours. Tablets may be chewed, swallowed with water or milk, or allowed to dissolve in the mouth.

**Supplied:** Bottles of 50, 100, 200 and 1000.

Winthrop

LABORATORIES • NEW YORK 18, NEW YORK

# reduces anginal attacks and fear of attacks

protects  
against pain  
by sustained  
coronary  
vasodilatation  
and control  
of complicating  
and triggering  
emotions

reduces fear of attacks  
reduces severity of attacks  
reduces frequency of attacks  
reduces dependence on nitroglycerin  
increases workload tolerance

Supplied: Tablets, vials of 50. Each tablet contains 200 mg.  
of meprobamate and 10 mg. of pentaerythritol tetranitrate.

## EQUANITRATE

Meprobamate and Pentaerythritol Tetranitrate, Wyeth



Philadelphia 1, Pa.

# *The Quality Of Greatness*

is rare in any human endeavor. When it appears, it may be perceived in various forms—as a work of art, a discovery, an idea, or an achievement of scientific inquiry. The outward form is incidental, but the intrinsic quality is readily recognized....

To partake of the quality of greatness, a therapeutic preparation must first of all achieve a degree of universality...the cumulative experience of thousands of physicians over a period of many years. From this experience, then, is born that unhesitating confidence which may be summed up in the term “drug of choice.”

## *Gantrisin*



ROCHE LABORATORIES  
Division of Hoffmann-La Roche Inc • Nutley 10 • N. J.

ROCHE—Reg. U. S. Pat. Off.

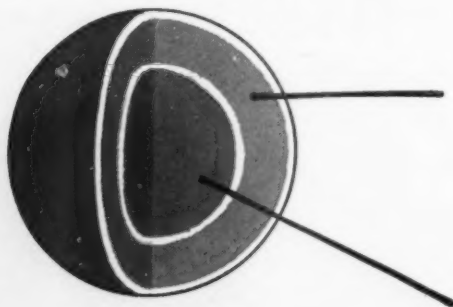
GANTRISIN®—brand of sulfisoxazole





## the complaint: "nervous indigestion"

**the diagnosis:** any one of several nonspecific gastrointestinal disorders requiring relief of symptoms by sedative-antispasmodic action with concomitant digestive enzyme therapy. **the prescription:** a new formulation, incorporating in a single tablet the actions of Donnatal and Entozyme. **the dosage:** two tablets three times a day, or as indicated.



*the formula: in the gastric-soluble outer layer:*

Hyoscyamine sulfate .....	0.0518 mg.
Atropine sulfate .....	0.0097 mg.
Hyoscine hydrobromide .....	0.0033 mg.
Phenobarbital (1/8 gr.) .....	8.1 mg.
Pepsin, N.F. ....	150 mg.

*in the enteric-coated core:*

Pancreatin, N.F. ....	300 mg.
Bile salts .....	150 mg.

# DONNAZYME<sup>TM</sup>



A. H. ROBINS COMPANY, INCORPORATED • RICHMOND 20, VIRGINIA



# PAIN Zactirin<sup>®</sup>

Ethoheptazine Citrate with Acetylsalicylic Acid, Wyeth

for everyday pain control . . .

for your many patients requiring  
potent analgesia but not an injected narcotic

*Proved by extensive evaluation<sup>1,2,3</sup> in 1998 patients in diverse areas of medicine and surgery, including:*

arthritis, bursitis, early metastatic carcinoma, fibrositis, grippe, herpes zoster, ligament strain, low back pain, menstrual pain, myalgia, myositis, neuritis, pleurisy, postoperative pain, postpartum pain, sciatica, trauma, dental pain

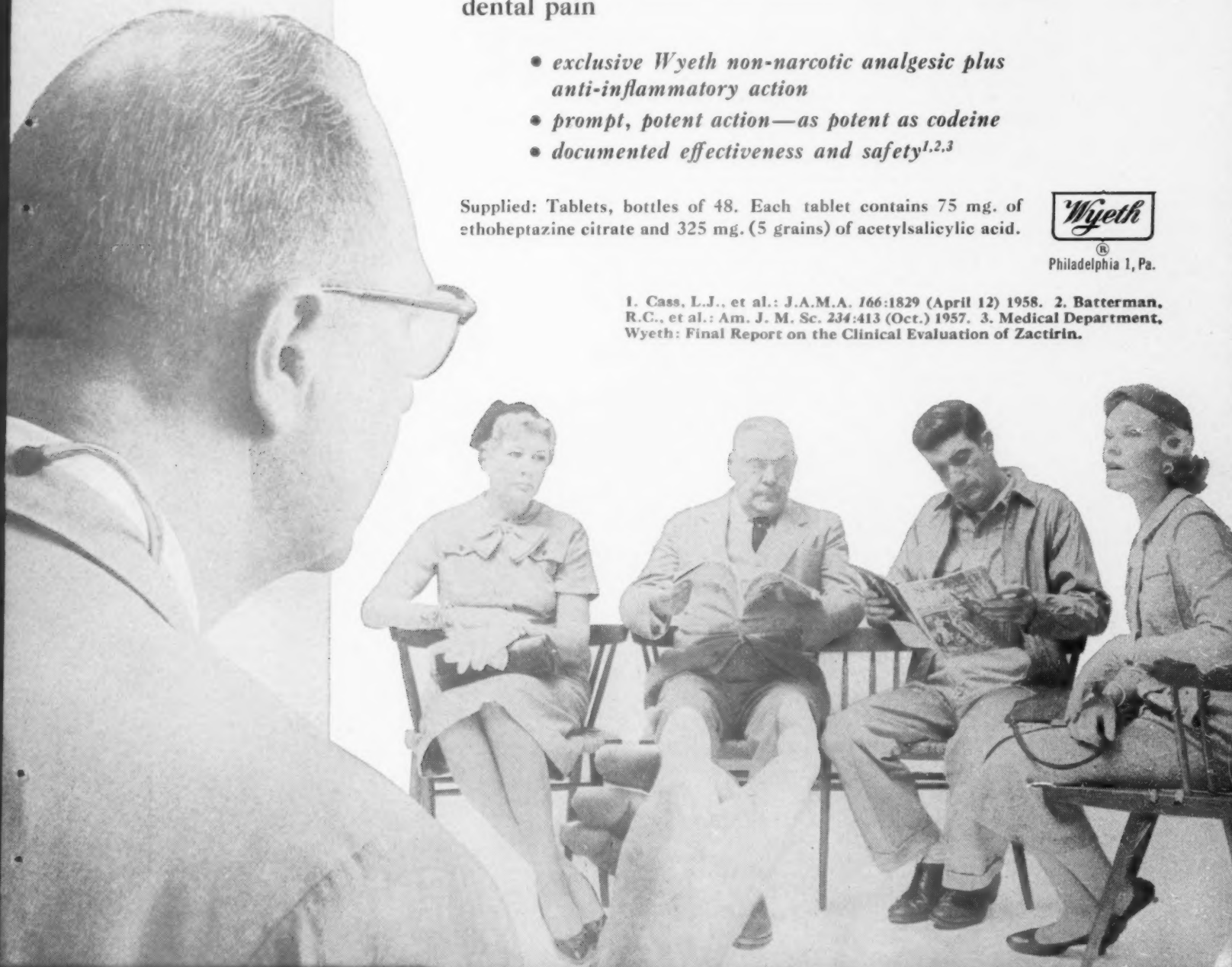
- *exclusive Wyeth non-narcotic analgesic plus anti-inflammatory action*
- *prompt, potent action—as potent as codeine*
- *documented effectiveness and safety<sup>1,2,3</sup>*

Supplied: Tablets, bottles of 48. Each tablet contains 75 mg. of ethoheptazine citrate and 325 mg. (5 grains) of acetylsalicylic acid.



Philadelphia 1, Pa.

1. Cass, L.J., et al.: J.A.M.A. 166:1829 (April 12) 1958. 2. Batterman, R.C., et al.: Am. J. M. Sc. 234:413 (Oct.) 1957. 3. Medical Department, Wyeth: Final Report on the Clinical Evaluation of Zactirin.



IN URTICARIA AND PRURITUS

**VISTARIL\***

HYDROXYZINE PAMOATE



## A PSYCHOTHERAPEUTIC ANTIHISTAMINE

(as designated by A.M.A. Council on Drugs, 1958)

**SPECIFIC ANTIHISTAMINIC ACTION** in the treatment of a variety of skin disorders commonly seen in your practice.

"While some of the tranquilizers are only partially effective as far as antiallergic activities are concerned...[hydroxyzine] has been found, by comparison, to be the most potent thus far..."<sup>1</sup>

"The most striking results were seen in those patients with chronic urticaria of undetermined etiology."<sup>2</sup>

### PLUS

**PSYCHOTHERAPEUTIC POTENCY** for the relief of anxiety and tension.

The psychotherapeutic effectiveness of hydroxyzine (VISTARIL) was confirmed in a series of 479 patients suffering from a wide variety of dermatoses, including atopic dermatitis, neurodermatitis, psoriasis, lichen planus, nummular eczema, dyshidrosis, pruritus ani and vulvae, and rosacea. "Adverse reactions were minimal."<sup>3</sup>

**RECOMMENDED ORAL DOSAGE:** 50 mg. q.i.d. initially; adjust according to individual response.

VISTARIL Capsules: 25 mg., 50 mg., 100 mg.

VISTARIL Parenteral Solution: 10 cc. vials and 2 cc. Steraject® Cartridges. Each cc. contains 25 mg. hydroxyzine (as the HCl).

### REFERENCES:

1. Eisenberg, B. C.: Clinical Medicine 5:897-904 (July) 1958.
2. Feinberg, A. R., et al.: J. Allergy 29:358 (July) 1958.
3. Robinson, H. M., et al.: So. Med. J. 50:1282 (Oct.) 1957.



Science for the world's well-being

**PFIZER LABORATORIES** Division, Chas. Pfizer & Co., Inc., Brooklyn 6, N. Y.

More  
than a  
tranquilizer

®TRADEMARK

# **SYMPTOMATIC RELIEF ...AND FAST**

*from the*

## **DRIP AND STUFFINESS**

*associated with*

## **COMMON COLD**

# **'FEDRAZIL'<sup>®</sup>**

**Sugar-coated Tablets**

**... contain an orally effective nasal decongestant  
combined with a good antihistamine**

**Dose: 2 tablets initially, then one every 3 or 4 hours as needed**

Each sugar-coated tablet contains: 'Sudated'<sup>®</sup> brand Pseudoephedrine Hydrochloride . . . 30 mg.

'Perazil'<sup>®</sup> brand Chlorcyclizine Hydrochloride . . . 25 mg.



**BURROUGHS WELLCOME & CO. (U.S.A.) INC., Tuckahoe, New York**

*enhanced progestational activity...*

# NORLUTIN<sup>®</sup>

(norethindrone, Parke-Davis)

*a potent oral agent, "effective in producing progestational changes in very low dosage."*<sup>1</sup>

**In childlessness:** NORLUTIN was used<sup>2</sup> in a group of women who had definite infertility problems and who had had substantial previous study and treatment. One or more studies of luteal function in each of these patients demonstrated the presence of some abnormality. Of 78 patients treated with NORLUTIN, 19 conceived. The investigators conclude that "...the new steroids [such as NORLUTIN] definitely do offer us a better chance of improving fertility in certain patients and of helping them achieve a pregnancy that otherwise might not occur. ...The incidence of side reactions with NORLUTIN was surprisingly low."<sup>2</sup>

## Results of therapy in infertility using NORLUTIN<sup>+</sup>

Number of patients treated	Number of pregnancies	Time of pregnancies	
78	19	First cycle	4
		Second cycle	7
		Third to sixth cycles	8

\*Adapted from Tyler & Olson<sup>2</sup>

**Indications for NORLUTIN:** conditions involving deficiency of progesterone, such as primary and secondary amenorrhea, menstrual irregularity, functional uterine bleeding, endocrine infertility, habitual abortion, threatened abortion, premenstrual tension, and dysmenorrhea.

**Packaging:** 5-mg. scored tablets (C.T. No. 882), bottles of 30.

**References:** (1) de Alvarez, R. R., & Smith, E. K.: *J.A.M.A.* 168:489, 1958. (2) Tyler, E. T., & Olson, H. J.: *Ann. New York Acad. Sc.* 71:704, 1958.

PARKE, DAVIS & COMPANY • DETROIT 32, MICHIGAN

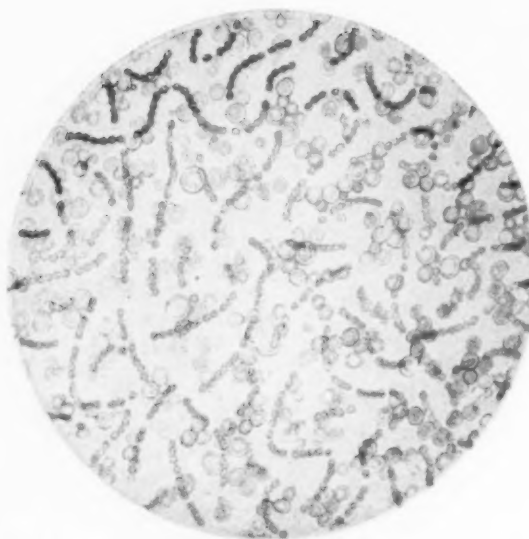
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*eradicate bacterial infection  
prevent monilial superinfection*



**COSA**—*natural potentiation with glucosamine  
for peak antibiotic serum levels*

**TETRACYCLINE**—*antibiotic activity against the broad range  
of susceptible organisms*

**NYSTATIN**—*antifungal protection against  
monilial superinfection*

## **COSA-TETRASTATIN®**

glucosamine-potentiated tetracycline with nystatin

### *capsules*

250 mg. glucosamine-potentiated  
tetracycline (Cosa-Tetracyn®) plus  
250,000 u. nystatin

### *oral suspension*

orange-pineapple flavored, 2 oz. bottle,  
each teaspoonful (5 cc.) contains  
125 mg. glucosamine-potentiated  
tetracycline (Cosa-Tetracyn®)  
plus 125,000 u. nystatin

**Pfizer** *Science for the world's well-being*

Pfizer Laboratories,  
Division, Chas. Pfizer & Co., Inc.  
Brooklyn 6, N. Y.

now...

*quinidine  
b.i.d.*

(one dose every 12 hours)



**QUINAGLUTE<sup>®</sup>**  
**DURA-TAB S.M.**

the only oral Sustained Medication\*  
quinidine gluconate, 5 gr.

for control of

## cardiac arrhythmias

- each dose of Quinaglute Dura-Tab S.M.<sup>®</sup> maintains uniform plasma levels up to 12 hours.<sup>1</sup>
- no night dosage needed.
- better absorbed and tolerated than quinidine sulfate.
- an unexcelled quinidine in premature contractions, auricular tachycardia, flutter, fibrillation.



PAGE 867

Dosage: For **conversion** of auricular fibrillation to normal sinus rhythm, in most cases, 2 Quinaglute Dura-Tab S.M. tablets 3 to 4 times a day, for 2 to 3 days.

For **maintenance** 1 to 2 tablets every 10 to 12 hours.

Supplied: Bottles of 30, 100 and 250.

samples, reprint and detailed literature.

**WYNN PHARMACAL**  
CORPORATION

5119 West Stiles Street  
Philadelphia 31, Pa.

1. Bellet, S., Finkelstein, D., and Gilmore, H.:  
A.M.A. Archives Internal Med. 100:750, 1957.

\*Patent Applied For

SEE US at BOOTH #63—AMERICAN COLLEGE OF CARDIOLOGY CONVENTION  
Benjamin Franklin Hotel, Phila., Pa.—May 25-29

# allergic SWELLING

## Dimetane<sup>®</sup> works

(PARABROMOYLAMINE MALEATE)


For your next patient with allergic swelling, itching or respiratory congestion associated with urticaria, Rx DIMETANE Extentabs<sup>®</sup> (12 mg.), Tablets (4 mg.), Elixir (2 mg./5 cc.) new DIMETANE-TEN Injectable (10 mg./cc.) or new DIMETANE-100 Injectable (100 mg./cc.). A. H. Robins Co., Inc., Richmond 20, Virginia  
Ethical Pharmaceuticals of Merit Since 1878





# B.I.D.

## ULCER CONTROL


all day 

NEW

# DARICON<sup>\*</sup>

oxyphencyclimine hydrochloride

## TABLETS

all night 

## patient comfort

**Natural Prolonged Action**—The action of DARICON, a more potent and better tolerated anticholinergic, is consistently prolonged because it has a unique chemical structure and is not dependent on "mechanical" means (e.g., special coating, adsorption on ion-exchange resin).

In addition to peptic ulcer, DARICON, is also indicated for other gastrointestinal disorders characterized by hypersecretion, hypermotility and spasm (e.g., functional bowel syndrome, chronic nonspecific ulcerative colitis and biliary tract disease).

**Dosage:** 10 mg. b.i.d. (morning and evening). **Supply:** Tablets, 10 mg., white, scored. Bottles of 60 and 500.

<sup>\*</sup>Trademark

 *Science for the world's well-being*

**PFIZER LABORATORIES**

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Brooklyn 6, N. Y.



EVEN REFRACTORY CASES RESPOND

in  
**childbirth**



**THORAZINE®**

brand of chlorpromazine

'Thorazine' as adjunctive therapy has four advantages:

1. reduces suffering
2. minimizes risk of respiratory depression
3. controls nausea and vomiting
4. relieves apprehension and agitation



Smith Kline & French Laboratories



Tremors no longer disrupt daily routine

**Cogentin**  
METHANESULFONATE (Benztropine Methanesulfonate)

For a more comfortable life  
 in **all** forms of  
 Parkinsonism



COGENTIN often means effortless performance of daily routine for patients with Parkinsonism—whether arteriosclerotic, postencephalitic, idiopathic, or tranquilizer-induced. COGENTIN “will counteract rigidity, contractures, frozen states and muscle cramps better than any current preparation.”<sup>1</sup> COGENTIN acts without causing drowsiness or foginess<sup>2</sup> and will even control resistant, major tremors in many patients,<sup>3</sup> thus assuring a more comfortable life. With COGENTIN to offset Parkinson-like symptoms, tranquilizer therapy may usually be continued without interruption or decrease in dosage.

**Dosage:** usual daily dose is 1 to 2 mg., with a range of 0.5 to 6 mg. Frequently, a single dose at bedtime is sufficient to control symptoms for 24 hours. In tranquilizer-induced Parkinsonism, the usual dosage is 1 to 2 mg. two or three times a day. When COGENTIN is used to offset the distressing Parkinson-like symptoms caused by tranquilizers, adequate therapy with these drugs may usually be continued. A decrease in dosage is rarely necessary.

Supplied: as quarterscored 2 mg. tablets in bottles of 100 and 1000.

1. Brock, S., Mod.: Bull. New York Acad. Med. 32:202, 1956.

2. Doshay, L. J.: Parkinsonism and Its Treatment, Philadelphia, J. B. Lippincott, 1954, pp. 87-88.

3. Doshay, L. J., Constable, K., and Zier, A.: Neurology 3:360, 1953.

COGENTIN is a trademark of Merck & Co., Inc.

Additional information on COGENTIN is available to physicians on request.



**Merck Sharp & Dohme**

DIVISION OF MERCK & CO., Inc., PHILADELPHIA 1, PA.

## AN AMES CLINIQUICK<sup>®</sup>

CLINICAL BRIEFS FOR MODERN PRACTICE

### THE CHANGE TO ORAL ANTIDIABETIC THERAPY

...does not reduce the need for attention to *diet*, *tests for sugar* in the urine, and *tests for acetone* in the urine...\*

\*Duncan, G. G.: Pennsylvania M. J. 61:46, 1958.

#### TEST DAILY FOR GLYCOSURIA

**COLOR-CALIBRATED  
CLINITEST<sup>®</sup>**  
BRAND Reagent Tablets

*the standardized urine-sugar test  
for reliable quantitative estimations—better control*

- established "plus" system covers entire critical range—includes  $\frac{3}{4}$  % (++) and 1% (+++)
- standard blue-to-orange spectrum long familiar to diabetics

available: CLINITEST Reagent Tablets • Bottles of 36 • Boxes of 24  
(Sealed in Foil) • CLINITEST Urine-Sugar Analysis Set

#### TEST DAILY FOR KETONURIA

**ACETEST<sup>®</sup>**  
BRAND Reagent Tablets

*specific for ketone bodies • no false positives*

- one drop of urine...one tablet...a few seconds detects both acetoacetic acid and acetone


available: ACETEST Reagent Tablets—  
bottles of 100 and 250.  
also available as "dip-and-read" test—  
KETOSTIX<sup>®</sup> Reagent Strips, bottles of 90.

**AMES**  
COMPANY, INC.  
Elkhart • Indiana  
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SQUIBB ANNOUNCES NEW

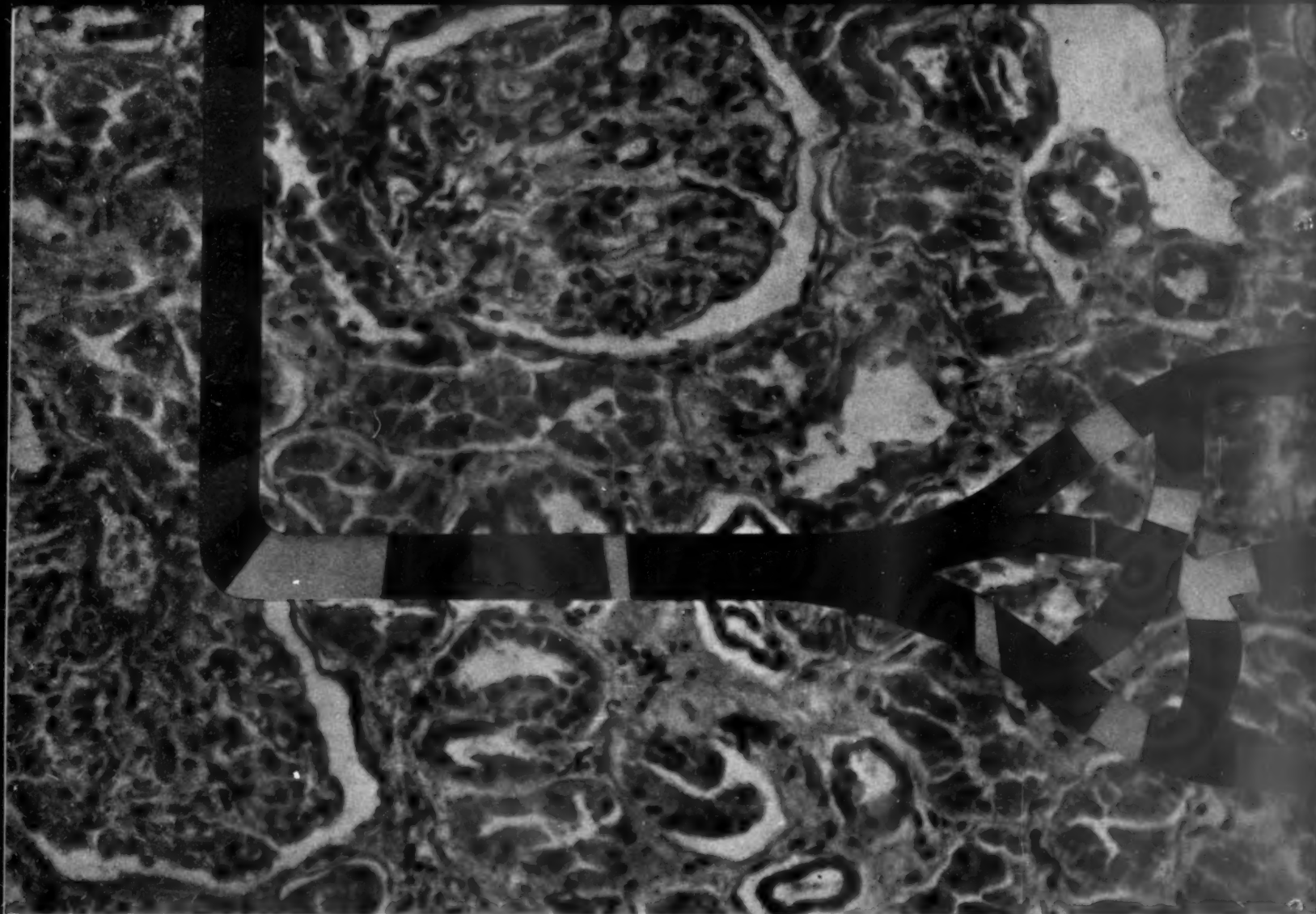
# RAUTRAX

a logical combination — Raudixin enhanced  
by an entirely new diuretic...Flumethiazide

SQUIBB

thus Squibb offers you greater latitude in solving the problem of

# HYPERTENSION



# RAUT

**for the treatment of hypertension...without**

Rautrax combines Raudixin with flumethiazide for control of all degrees of hypertension. Clinicians report it safely and rapidly eliminates excess extracellular sodium and water without potassium depletion.<sup>1,2,3</sup> Through this dependable diuretic action of flumethiazide, the clinical and subclinical edema—so often associated with cardiovascular disease—is rapidly brought under control.<sup>2,3,4,5</sup> Flumethiazide also potentiates the antihypertensive action of Raudixin. By this unique dual

action, a lower dosage of each ingredient effectively maintains safe antihypertensive therapy.

**Diuresis without serum electrolyte imbalance**

Flumethiazide—the new, safe nonmercurial diuretic—rapidly achieves its diuretic effect without causing appreciable plasma electrolyte imbalance.<sup>1,2,3</sup> Potassium loss is less than with other nonmercurial diuretics.<sup>1</sup> Moreover, the inclusion of supplemental potassium chloride in Rautrax provides added protection against potassium



# RAX

**RAUDIXIN**

SQUIBB STANDARDIZED  
WHOLE ROOT RAUWOLFIA SERPENTINA

**FLUMETHIAZIDE**

**POTASSIUM CHLORIDE**

## fear of significant potassium depletion<sup>1, 2, 3</sup>

and chloride depletion in the long-term management of the hypertensive patient.

Sodium and water retention is rapidly relieved,<sup>2</sup> and once the fluid balance has been brought within normal limits, continued administration of Rautrax does not appreciably alter the normal serum electrolyte pattern.

### **Control of hypertension with fewer side effects**

Raudixin—a cornerstone on which to build a therapeutic regimen for control of hypertension.

### **RAUTRAX... GREATER LATITUDE IN SOLVING THE PROBLEM OF HYPERTENSION**

- Prompt, safe antihypertensive effect — by the complementary action of Raudixin and flumethiazide
- Less potassium loss than with other nonmercurial diuretics<sup>4</sup>
- No loss in effectiveness after continued administration
- No influence on blood urea nitrogen, blood count or other hematologic values<sup>5</sup>
- Fewer and less severe side effects<sup>6, 7</sup>
- Less need for severe restriction in sodium intake
- Gout, purpura, or allergic reactions not reported

**SQUIBB**





**RAUDIXIN PLUS AN ENTIRELY NEW DIURETIC...FLUMETHIAZIDE**

**A NATURAL COMPANION**

**TO FAMOUS RAUDIXIN TO HELP SOLVE THE PROBLEM...**

## **HYPERTENSION**

**Dosage:** 2 to 6 tablets daily in divided doses initially; may be adjusted within range of 1 to 6 tablets daily in divided doses. **Note:** In hypertensive patients already on ganglionic blocking agents, veratrum and/or hydralazine, the addition of Rautrax necessitates an immediate dosage reduction of these agents by at least 50%. A similar reduction is also necessary when these ganglionic blocking agents are added to the Rautrax regimen.

**Literature available on request.**

**Supply:** Capsule-shaped tablets each providing 50 mg. Raudixin, 400 mg. flumethiazide, and 400 mg. potassium chloride, bottles of 100.

**References:** 1. Moyer, J.H., and others: *Am. J. Cardiol.*, 3:113 (Jan.) 1959. • 2. Bodi, T., and others: To be published, *Am. J. Cardiol.*, (April) 1959. • 3. Fuchs, M., and others: *Monographs on Therapy*, 4:43 (April) 1959. • 4. Montero, A.C.; Rochelle, J.B., III, and Ford, R.V.: To be published. • 5. Rochelle J.B., III; Montero, A.C., and Ford, R.V.: To be published. • 6. Montero, A.C.; Rochelle, J.B., III, and Ford, R.V.: To be published. • 7. Doffermyer, L.R.; Byrd, C.W., and Lilly, W.H.: *North Carolina M.J.* 10:430 (Oct.) 1958.

**SQUIBB**



**Squibb Quality — the Priceless Ingredient**





*when every  
complaint  
reacts on  
her stomach*

**TO HELP HER G.I. TRACT—AND HER—REGAIN THEIR COMPOSURE**

# BUTIBEL<sup>®</sup>

*antispasmodic-sedative*

"quiets down" the queasy, spastic colon—through the gentle sedation of BUTISOL Sodium<sup>®</sup> butabarbital sodium 15 mg. and the relaxing action of *natural* extract of belladonna 15 mg. The central and peripheral effects of BUTIBEL are of approximately equal duration—no overlapping sedation or inadequate spasmolysis.

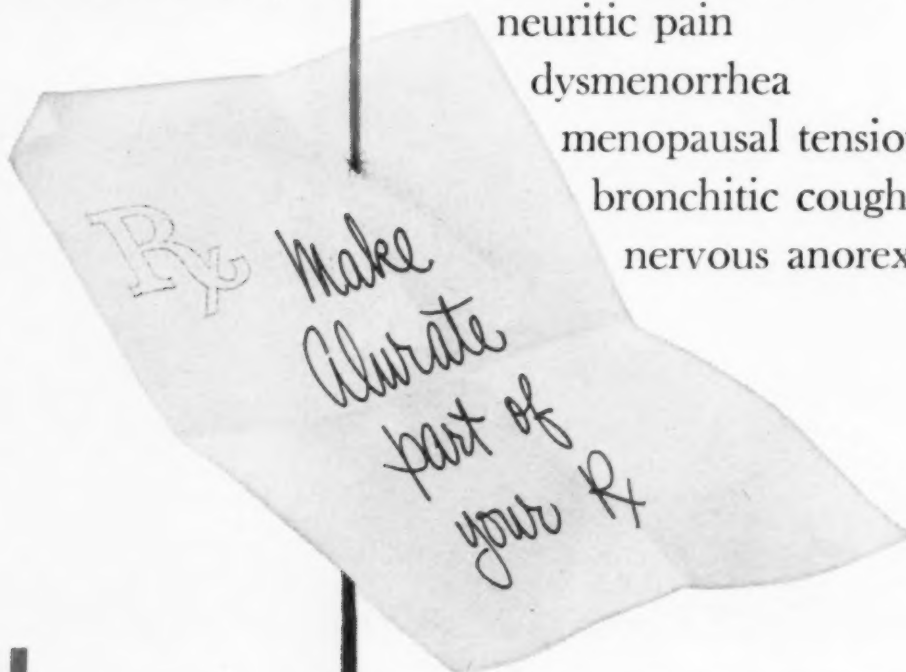
**BUTIBEL TABLETS • ELIXIR • PRESTABS<sup>®</sup> BUTIBEL R-A**  
(Repeat Action Tablets)

**McNEIL**

McNEIL LABORATORIES, INC.  
PHILADELPHIA 32, PA.

# When sedation is part of your treatment

as in gastrointestinal spasm  
neuritic pain  
dysmenorrhea  
menopausal tension  
bronchitic cough  
nervous anorexia



## Alurate®

Each teaspoonful (5cc) contains 40 mg of  
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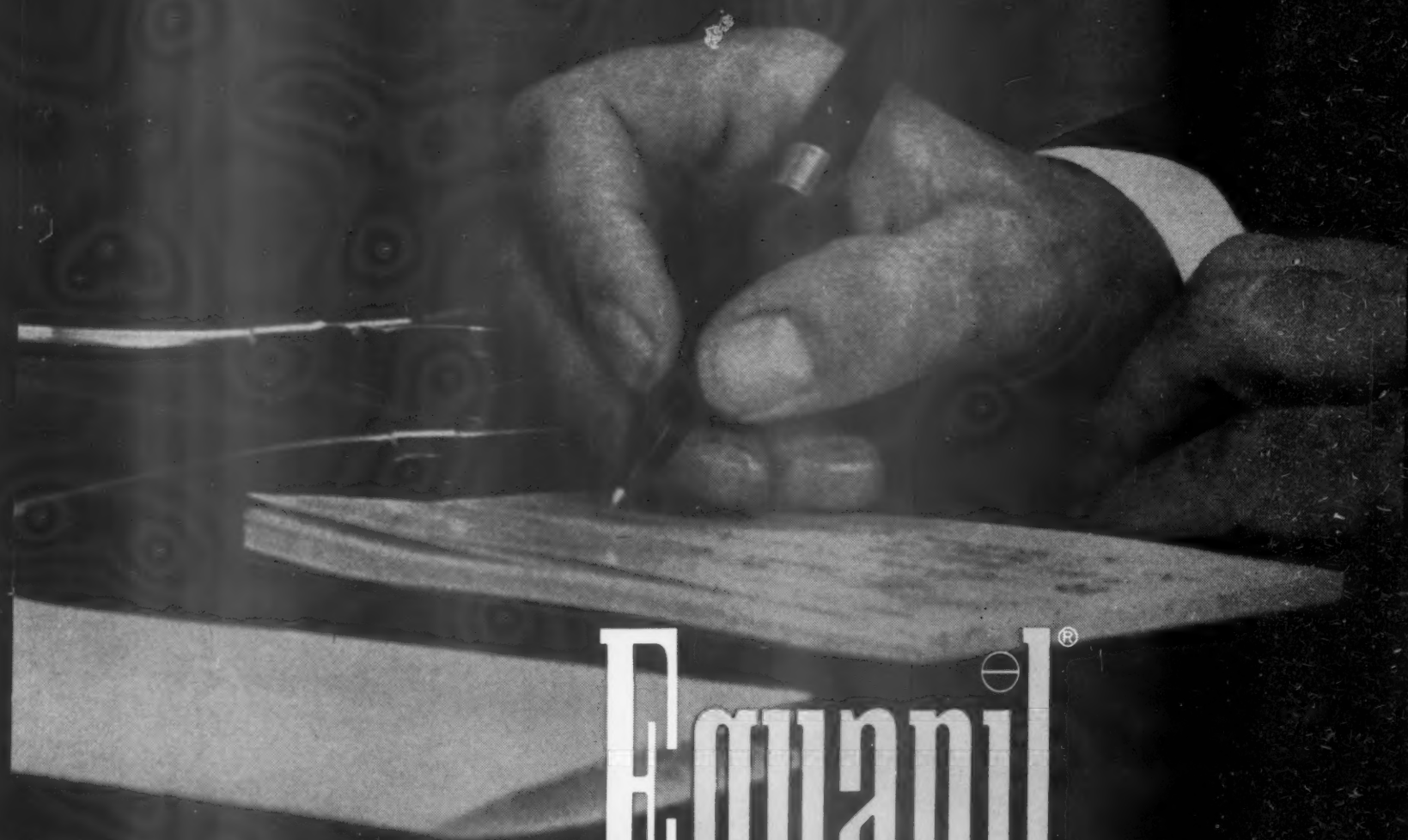
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1. Coleman, S. S.: *Am. J. Surg.* 97:43 (Jan.) 1959.
2. Richardson, M. E.: *J. Am. Osteop. A.* 57:562 (May) 1958.
3. Mason, M. L.: *Northwest Med.* 57:1439 (Nov.) 1958.

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# The American Journal of Medicine

VOL. XXVI

MAY, 1959

No. 5

## Foreword

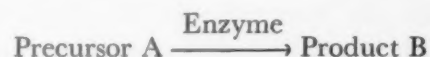
### A Current View of Metabolic Errors

OVER the past generation it has become increasingly apparent that a large fraction, possibly the majority, of the protein species in the organism serve specific catalytic functions, are, in other words, enzymes. To this complement of enzymes, additions may from time to time be made, either by induction or by genetic mutation. From this complement of enzymes, deletions may also occur, either by mutation or by some oblitative somatic process. The integrated operation of the metabolic process is dependent upon the integrity of this complement of enzymes.

The list of "inborn errors of metabolism," originally tabulated by Garrod, has become longer over the years. Considerable insight into the mechanisms underlying these and other metabolic defects has accrued. The philosophy, stemming from interpretations of microbiological studies, has developed wherein gene, enzyme and metabolic step are assumed to be in unit ratio, i.e., the presence of each enzyme is determined by the integrity of a particular gene, and the occurrence of each metabolic step depends upon a particular enzyme. Whereas in no instance, to this writer's knowledge, has a disease in man been clearly attributed to the abnormal presence of an enzyme normally absent in the organism, in a growing number of cases it has become quite clear that absolute lack or relative deficiency of an enzyme normally represented does occur and this may produce

clinically significant disease.\* If this enzyme lack is genetically transmitted, the defect is presumed to be due to a discrete mutational event involving a single gene. Since most enzymes exhibit fairly high catalytic specificity, the lack of an enzyme is generally reflected, metabolically, in the failure of a single biochemical step in one or another sequence. To diseases presumed to arise by such a sequence of events the name "molecular disease" has been given. The consequences of such a metabolic block may be large or small, depending upon the location of the block in the general metabolic scheme. A vascular block, thrombotic or embolic, if in an area of abundant anastomoses, is less traumatic than if in an area in which anastomoses are scarce or non-existent. Analogously, in the metabolic situation, if there are collateral channels leading to the product and away from the precursor, a blocked reaction may be perfectly compatible with normal survival.

Several situations may be envisioned and for most of these, today, examples may be cited. Consider the reaction:



\* The positions of the important metabolic blocks encountered in human carbohydrate metabolism are indicated by Burns (*Am. J. Med.*, 26: 740, 1959; see Fig. 6). Several of these blocks are discussed in detail in the ensuing contributions.

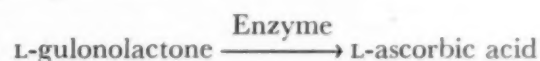
The genetic loss of capacity to generate the requisite enzyme may result in disease only if precursor A is in some wise toxic or if product B is required in some important biochemical reaction. If neither of these conditions is fulfilled, the enzyme can be lost with impunity. Such a situation is encountered in the uricase reaction wherein A = uric acid and B = allantoin. Except in the gouty person, uric acid is not notably toxic and has, besides, an alternative and usually adequate route of disposal, notably, renal excretion. Allantoin appears to be needed in no important process. Loss of uricase has therefore been tolerated in higher apes and man with no apparent impairment of survival opportunity and so far as is known, the entire species *Homo* suffers from this deficiency.

A similar situation obtains in the case of deficiency of the enzyme TPN-xylitol dehydrogenase [7]. In this instance the precursor A = L-xylulose and the product B = xylitol. As in the foregoing example, the precursor is not remarkably toxic and also is readily excreted in the urine. The product may or may not be important biochemically but since at least one other independent route is available for its biogenesis, namely from D-xylulose, failure of this reaction is of no significant consequence in this regard. Other than the appearance of L-xylulose in the urine, the genetic lack of TPN-xylitol dehydrogenase from mammalian tissues results in no signs or symptoms, and essential pentosuria is classified as a disease by courtesy only. This enzyme lack is but rarely manifest in man in contrast to uricase deficiency, which appears to be universal.

The association of overt disease with genetic enzyme deficiency is best seen in those situations wherein precursor A is toxic and alternate routes for its disposal are inadequate. Such is the probable situation in alcaptonuria, in oligophrenic phenylpyruvicaciduria, and possibly in many other diseases. A well documented example, with reference to the present seminar [2], is galactosemia. The genetic failure to synthesize phosphogalactose uridyl transferase affects adversely the reaction wherein precursor A, galactose 1-phosphate, goes to product B, uridine diphosphate galactose. In this instance, whereas B can arise by other pathways, alternate routes

of disposal of A are limited, and its accumulation in tissues is distinctly deleterious. Pathologic consequences of this accumulation in brain, lens and liver are most severe in childhood. When, at or about adolescence, an alternate route for disposal of galactose 1-phosphate becomes more prominent, the metabolic consequences of transferase lack may diminish somewhat.

Yet another type of enzyme deficiency and metabolic block which leads to disease is one in which the normal reaction yields an indispensable product. When this missing reaction product, B, can be adequately replaced by dietary supplementation, the disease in question is usually classified as a nutritional deficiency, and until recently the underlying enzymic deficiency has not been generally recognized. A case in point [3] is the reaction occurring in all known mammalian species except guinea pigs, man and other primates:



The lack of this enzyme would be lethal to man were it not that the missing reaction product is widely distributed in the natural diet and is satisfactorily absorbed therefrom. Toxicity of the precursor does not appear to be involved here, and the fact that it does not accumulate in man suggests other routes for its disposal.

It seems probable that ultimately the role of each of the amino acids and vitamins known to be essential in the human dietary will be analyzed in terms of a single enzyme defect. Certainly in scurvy it would appear reasonable, as in galactosemia, to list the disease as one arising from a mutation which resulted in the loss of an enzyme present in a more nearly autotrophic ancestral stock. Since the genetic defect leading to scurvy is apparently universal in man, it is indeed fortunate that replacement therapy is readily available. Feeding of ascorbic acid does not however, correct the underlying enzyme defect, any more than vitamin B<sub>12</sub> administration corrects the underlying malfunction of gastric mucosa in Addisonian anemia. By analogy, we may say that all mankind has scurvy, but for most of us, fortunately, the disease is in remission.

Undoubtedly the most important clinical disturbance of carbohydrate metabolism is diabetes. The day may come when we can point with

<sup>3</sup> BURNS, J. J. Biosynthesis of L-ascorbic acid; basic defect in scurvy. *Am. J. Med.*, 26: 740, 1959.

<sup>1</sup> TOUSTER, O. Pentose metabolism and pentosuria. *Am. J. Med.*, 26: 724, 1959.

<sup>2</sup> ISSELBACHER, K. J. Galactose metabolism and galactosemia. *Am. J. Med.*, 26: 715, 1959.

certainty to one enzymatic step, as we can in galactosemia or in scurvy, and say, "Here is the locus of the primary process that is insulin-responsive." At the present time no such statement is possible. Probably even more remote are the answers to the questions, "What is the enzymatic defect which limits the insulin production in  $\beta$ -cells of certain individuals? What

is the defect leading to excessive production of insulin antagonists or insulin-destroying agents?" These are among the questions to which answers must be sought.

DEWITT STETTEN, JR.  
*National Institutes of Health  
National Institute of Arthritis and Metabolic Diseases  
Bethesda 14, Maryland*

# Symposium on Disorders of Carbohydrate Metabolism

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## On the Nature of the Metabolic Defect(s) in Diabetes<sup>\*</sup>

JAMES B. FIELD, M.D.

*Bethesda, Maryland*

**D**IABETES mellitus has been defined as a disease characterized by a disturbance of carbohydrate metabolism in which the normal balance of the regulatory factors is altered [1]. A corollary to this definition is that in the diabetic person there is a deficiency of insulin in relation to the body's need, since insulin is unique in its ability to decrease the blood sugar. This deficiency may occur in the presence of either reduced or normal secretion of insulin by the pancreas and can be considered to be the essential cause of the metabolic derangements which are observed in the diabetic patient. In addition to the carbohydrate abnormality, the untreated diabetic patient suffers from alterations in fat and protein metabolism, which might represent either primary or secondary defects.

In any discussion concerning the nature of the metabolic defect(s) in diabetes, the relative roles of both overproduction and underutilization of glucose must be considered. Closely related to this problem is the consideration of the action of insulin on the various tissues of the body, discussed in detail elsewhere [2]. It is fully realized that the aspects mentioned do not encompass all phases of the metabolic derangement in diabetes, but because of space limitations they have been selected as representing the more important facets of the problem. It should be emphasized at the outset that much of the experimental work which has been done to elucidate these defects has been carried out in animals, and the conclusions drawn might not necessarily be applicable to the problem of diabetes in man.

All patients with persistent hyperglycemia

have been included together under the clinical definition of diabetes [3]. Such a definition may be an oversimplification and tends to obscure the fact that different metabolic abnormalities may all happen to produce hyperglycemia. Clinically at least, two different types of diabetic patients have been delineated [4-6]. On the basis of the response to a glucose and insulin-glucose tolerance test, Himsworth divided diabetic patients into insulin-sensitive and insulin-insensitive types [4,5]. He suggested that the essential difference between the two types was in the different rate at which insulin action was apparent, rather than in its total effect. In the insulin-sensitive patient, diabetes resulted from a lack of insulin, whereas in the insulin-insensitive type it was due to diminished responsiveness to insulin. The insulin-sensitive diabetic patient tended to be thin, easily developed ketosis following insulin withdrawal, usually developed the disease before the age of thirty, and frequently manifested a certain lability in the blood sugar on insulin therapy. Synonymous terms for this type of patient include labile, brittle, growth-onset, juvenile, and insulin-deficient diabetic. On the other hand, the insulin-insensitive patient usually developed diabetes after the age of forty, was obese, and did not develop ketosis readily when insulin was discontinued. These patients could often be satisfactorily regulated by weight reduction and diet alone. They have also been referred to as stable, mild, maturity-onset, adult, or lipoplethoric diabetics. However, although the response to insulin seemed to divide the diabetic patients tested into two distinct

<sup>\*</sup> From the Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland.



groups, the correlation with clinical criteria was not always perfect [5]. Lawrence proposed a similar clinical classification of diabetes [6] and suggested that the defect in the lipoplethoric diabetic patient was an inability of overfilled fat depots to convert glucose to fat rather than an absolute insulin deficiency [7]. This explanation is somewhat difficult to reconcile with the observation that carbohydrate tolerance usually can be restored in this type of diabetic patient by proper diet and weight loss, even though the latter may be minimal [8].

Some experimental evidence corroborating the clinical distinction of two types of patients was provided by assays of plasma for insulin-like activity. Bornstein and Lawrence reported that the insulin-like activity of plasma from normal subjects averaged 0.34 milliunits/ml., while plasma from untreated patients with growth-onset diabetes was devoid of such activity [9]. Nearly normal amounts of insulin-like activity were detected in plasma from untreated patients with lipoplethoric diabetes. These results were confirmed by Vallance-Owen et al. who, indeed, found greater than normal levels of insulin-like activity in the obese diabetic patient [10]. Wright also has reported normal plasma insulin-like activity in the obese diabetic patient, while Willebrands et al. found normal levels in both types of diabetic patients [11,12].

Histologic examination of the pancreas provided further support for such a classification of diabetic subjects [13]. In a large number of patients with maturity-onset diabetes, present day methods of histologic examination of the pancreas fail to reveal significant abnormalities of the islet cells [13], although Maclean and Ogilvie reported a reduction in the proportion of  $\beta$  cells and a decrease to approximately half in the weight of the  $\beta$  cells [14]. In contrast, hyalinization with degranulation of the  $\beta$  cells is the rule in the patient with growth-onset diabetes and the reduction of the weight of the  $\beta$  cells is striking [13,14]. These histologic findings correlate well with the extractable insulin content of the pancreas [13-16]. While the extractable insulin content of the pancreas of obese patients with diabetes averaged 50 per cent of control values, there were numerous instances in which normal amounts were present. Markedly reduced amounts of extractable insulin were consistently present in the patients with the growth-onset type of diabetes. The relationship between

the amount of extractable insulin in the pancreas and the amount of  $\beta$  cell granulation was more constant than that with the frequency of islets.

A further metabolic distinction between these two types of diabetic patients has been proposed by Smith and Taylor [17]. These investigators noted a normal rise in the plasma pyruvate level following administration of glucose in the patient with maturity-onset diabetes, but not in the patient with growth-onset type of diabetes. However, the data of Butterfield and Thompson do not confirm this difference [18].

Despite the clinical and experimental evidence suggesting two different types of diabetes in man, there are many patients in whom this distinction is not so evident [5]. If there is a group of diabetic patients who have normal levels of circulating insulin-like activity in the plasma and near normal amounts of extractable insulin in the pancreas, the cause of their relative insulin deficiency and diabetes remains unexplained. DeWesselows and Griffiths reported that injection of plasma from obese diabetic patients into rabbits caused a marked diminution in the hypoglycemic response to insulin whereas plasma from normal subjects and those with juvenile diabetes had no such effect [19]. Subsequent investigation did not confirm the presence of a possible insulin antagonist in this type of diabetes [5,10,20].

However, a role for non-hormonal insulin antagonists has recently been suggested as a cause of the relative insulin deficiency in some cases of diabetes in man [21]. Vallance-Owen et al. were unable to demonstrate an effect of insulin when it was added to plasma from patients with growth-onset diabetes at a time when they were hyperglycemic [10]. No such antagonism was present in these patients when the blood sugar was normal. More recent experiments have indicated that although the antagonist was in the albumin fraction of serum proteins, it did not appear to be albumin itself [22,23]. Similar insulin antagonism was also present in albumin fractions obtained from non-diabetic subjects, but to a lesser extent [22]. The absence of this antagonist from plasma albumin fractions obtained from hypophysectomized persons suggested a possible pituitary origin [23]. A circulating insulin antagonist has also been reported in the pancreatectomized cat [24]. In this situation, the antagonist also disappeared following hypophysectomy or adrenalectomy and could be restored following several days of

administration of growth hormone and hydrocortisone. In the cat, the antagonist appeared to be in the globulin fraction of plasma. Field, Tietze and Stetten have reported a circulating insulin antagonist in the serums of some patients during diabetic acidosis [27]. This antagonist was observed in subjects who had not received previous insulin therapy and were not known to be diabetic until their episode of acidosis. By serum protein electrophoresis, it was possible to localize the antagonist in the  $\alpha_1$  globulin fraction. Although the antagonist described by Vallance-Owen et al. appeared to reside in the albumin fraction, most methods of protein fractionation are not precise enough to rule out the possibility of some cross-contamination and therefore, to date, there is no convincing evidence to indicate that these two substances represent different entities. Some of these observations have been confirmed [25], but Groen et al. were unable to demonstrate insulin antagonism in several cases of diabetic acidosis. [26]. It is too early to assess what role, if any, such antagonists might play in the etiology of insulin deficiency in the diabetic patient.

Berson et al. [27] have demonstrated an insulin-binding protein in the inter  $\gamma$ - $\beta$ -globulin zone in the plasma of diabetic patients who had received insulin therapy for several months. Since prior treatment with insulin appears to be a prerequisite for the appearance of this protein, it is most unlikely that it is of any etiologic importance in diabetes. The insulin antagonist present in some cases of chronic insulin resistance seems to be an antibody to heterologous insulin and probably cannot be considered a primary cause of insulin deficiency [28-30]. A  $\beta$ -lipoprotein substance has been described in the plasma of severely-alloxanized diabetic rats which was capable of inhibiting glucose uptake by normal rat diaphragm [31-33]. A possible relation to the pituitary and adrenals has been suggested, but since no similar factor has been described in the plasma of human subjects with diabetes, its role must remain problematic.

In addition to its spontaneous occurrence, diabetes is frequently encountered as part of another endocrine disorder such as acromegaly or Cushing's disease [1,34,35]. Perhaps in these situations the explanation for the relative insulin deficiency is more apparent although the precise nature of the effect of the pituitary and adrenal cortical hormones on carbohydrate metabolism is not completely understood. Since this subject

has been extensively reviewed recently [36], the present discussion will be limited to the relationship between growth hormone and diabetes in man.

Growth hormone appears to have both a stimulatory and inhibitory effect on glucose utilization, but in the whole animal the stimulatory effect predominates. In addition, the hormone appears to increase hepatic glucose production and pancreatic insulin secretion, and to decrease the hypoglycemic response to injected insulin [36]. The insulin-like activity of plasma from subjects with acromegaly, both with and without diabetes, has been reported to be elevated, using the rat hemidiaphragm technic [11, 37,38], and absent using the alloxan-diabetic hypophysectomized adrenalectomized rat [9]. Recently an insulin antagonist has been reported in the plasma of some acromegalic patients with diabetes [39]. Houssay concluded that insulin resistance in the dog resulting from treatment with anterior pituitary extracts resided in the tissue since it was not modified by replacement of 85 per cent of the blood volume with normal dog blood [1]. Although it has not been conclusively established, it is generally assumed that growth hormone and the principal "diabetogenic factor" from the anterior pituitary are synonymous [40,41]. Administration of human growth hormone preparations to a hypophysectomized non-diabetic patient resulted in a decrease in insulin sensitivity while ketosis and acidosis were produced in a hypophysectomized diabetic patient [42]. Several earlier workers have postulated a role for the anterior pituitary in the etiology of diabetes in man [1,19,43], and White reported that 87 per cent of 303 diabetic children were overweight prior to the onset of their disease [44]. In this group of children, the average bone age was advanced eighteen months. However, the observation of Young that crude preparations of growth hormone which were diabetogenic in the adult cat and dog did not produce diabetes in the kitten or puppy cast some doubt upon the influence of growth hormone in the etiology of juvenile diabetes [45]. At present, except in cases of acromegaly, there is very little evidence to implicate excessive production of growth hormone as responsible for the insulin deficiency in spontaneous diabetes [35,46].

The role of the adrenal oxycorticosteroids in carbohydrate metabolism has been extensively studied and recently reviewed [36]. Adrenal steroids stimulate gluconeogenesis and increase



the glucose output by the liver [47], but their effect on glucose utilization is controversial [36]. Wilson et al. have suggested that administration of adrenal steroids to the non-diabetic subject produces a compensatory increase in the insulin secretion by the pancreas [48], but it must be assumed that when diabetes results from such treatment a relative insulin deficiency exists. Despite the frequent occurrence of diabetes in patients with Cushing's disease, there is very little evidence that increased adrenal cortical activity is important in spontaneous diabetes. Becker et al. have reported increased urinary excretion of oxycorticosteroids in some diabetic patients [49], but most other investigators have found the values either normal [50-52] or low [53,54]. A reduced adrenal cortical response to the stress of surgery has been noted in some diabetic patients [55]. The response to a test injection of adrenocorticotropin was found to be normal in a group of diabetic patients [52].

Relative insulin insufficiency could also result from increased destruction of insulin. Mirsky has described an insulin-inactivating system, insulinase, which is present in most tissues of the body, especially in the liver and kidney [56]. Although insulinase has not been prepared in a highly purified state, it does not appear to be completely specific for insulin [57,58]. An increase in insulinase activity has been reported in liver tissue from patients with diabetes mellitus as compared with that from non-diabetic subjects [56]. However, insulin appears to be protected from degradation by the liver in the presence of plasma from diabetic patients who have been treated with insulin [59]. It is too early to know what role, if any, this system plays in diabetes in man.

Although we have considered most of the possible reasons for insulin deficiency, its cause remains obscure in a majority of diabetic patients. Regardless of the reason for the insulin deficiency, the end result—hyperglycemia and impaired carbohydrate tolerance—is the same, even though produced, perhaps, by different mechanisms. We shall now turn our attention to the metabolic derangements which are a consequence of the insulin deficiency. For many years, there were conflicting views as to whether diabetes mellitus in man resulted from underutilization of glucose by peripheral tissue or overproduction of glucose by the liver. The earlier work relating to this problem has been summarized by Soskin and Levine [60].

Just as the cause for the insulin deficiency may vary in the different types of clinical diabetes, so the consequence of this might have different expressions in the various types of diabetic patients. The arteriovenous difference in glucose concentration has been found to be decreased in diabetic patients as compared with that in non-diabetic subjects but is restored toward normal by the administration of insulin [61,62]. Evidently this effect of insulin is not observed uniformly in all diabetic persons [14]. In the insulin-insensitive patient, the A-V glucose difference was found to be negative in fasting subjects but was slightly positive thirty minutes after the ingestion of glucose [62]. The administration of insulin with the glucose did not result in a further increase in the A-V glucose differences. More recently, Zierler and Andres observed decreased glucose uptake by muscle in diabetic subjects and a diminished response to insulin even in patients who had not received prior insulin treatment [63]. Their experiments did not permit them to draw any conclusions as to whether the resistance to insulin resided in the tissue itself or was due to a circulating factor such as described by Vallance-Owen et al. [10]. Decreased glucose utilization was also found in hemidiaphragms from alloxanized diabetic rats with fasting blood sugars over 300 mg. per cent [64]. The hexokinase activity of extracts of muscle from both alloxan-diabetic rats and human diabetic patients appears to be normal [64,65]. Although the evidence cited indicates that there is a defect in the peripheral utilization of glucose in diabetes, and Soskin and Levine noted that at any particular blood sugar level the diabetic dog utilized less sugar than the normal dog, they concluded that at high blood sugar levels, the diabetic subject can utilize as much sugar as the normal subject at lower levels [66]. These conclusions have been challenged [67] but have been recently confirmed by the studies of Butterfield et al. [68]. These investigators reported that in the diabetic subject there is a raised threshold for glucose in the peripheral tissues, but that once this threshold is exceeded glucose utilization proceeds at a normal rate. This increased threshold was reduced toward normal by the administration of insulin [68].

The use of radioactive glucose has made it possible to estimate the relative roles of both underutilization and overproduction as contributing factors to the hyperglycemia of diabetes. Shreeve et al. divided diabetic patients into

"stable" and "labile" types and found 24 per cent of expired carbon dioxide was derived from glucose in the former, and an average of 18 per cent in the latter [69]. The average for normal subjects was 30 per cent and was significantly greater than the figure for the labile but not for the stable diabetic subjects. Although the glucose space was not significantly greater in either group of diabetic patients as compared with that in normal subjects, the size of the glucose pool was larger as a consequence of the hyperglycemia. Five of the diabetic patients, two stable and three labile, had turnover rates of glucose which exceeded those found in normal subjects. There was also no clear-cut distinction between the two groups in regards to the fate of the glucose utilized. From their data these authors concluded that in certain human diabetic subjects in whom ketosis follows insulin withdrawal there is a decreased rate of glucose oxidation as well as an increased rate of glucose production, primarily from the liver. In diabetic subjects in whom ketosis does not develop after insulin withdrawal the observed rate of oxidation at hyperglycemic blood levels was equal to that of the euglycemic normal control subject even in the absence of exogenous insulin. However, Shreeve et al. were careful to point out that the normal rate of glucose oxidation in the patient with stable diabetes at the time of the study when the blood sugar was elevated does not rule out antecedent impairment of oxidation as a contributing cause to the hyperglycemia.

Similar experiments in diabetic animals also have indicated both underutilization and overproduction of glucose as factors responsible for diabetic hyperglycemia [70-72]. It should be emphasized again that in none of these experiments was it possible to determine whether the augmented production of glucose by the liver was a primary defect causing the hyperglycemia or whether it was compensatory to promote more glucose utilization by the peripheral tissues [66]. Feller et al. found that glucose turnover in the alloxan-diabetic rat was twice that of the normal animal [70]. The rate of glucose oxidation in the diabetic animals was somewhat less than normal, and the authors concluded that this was not the major metabolic defect in the alloxan-diabetic rat. The opposite conclusion was reached by Stetten et al. who believed that the small increase above normal in the rate of glucose formation was not sufficient to support the view that overproduction of glucose was the main defect

in alloxan diabetes [71]. Both underutilization and overproduction of glucose have been observed in the diabetic dog [72].

The studies of Shreeve et al. suggest that the relative importance of overproduction and underutilization of glucose might vary with the clinical type of diabetic patient [69]. Over twenty years ago it was suggested that the defect in the stable type of diabetes was the inability of insulin to promote storage of sugar in the liver [62]. It has been possible to obtain some information on this point by catheterization of the hepatic vein [73-74]. In a group of forty-three diabetic patients deprived of insulin for at least forty-eight hours before study, Bearn et al. found no significant increase in hepatic glucose output as compared with that in normal subjects, even though the diabetic subjects were hyperglycemic [73]. Their conclusion that the peripheral utilization of glucose must also be normal, but at a higher blood sugar concentration, is consistent with earlier observations [66,74]. However, they considered the evidence to suggest strongly that overproduction of glucose was not the cause of diabetic hyperglycemia. Following insulin injection there was an immediate decrease in hepatic glucose production in both diabetic and normal subjects, but the response in the individual diabetic subjects varied widely. While the decrease in 90 per cent of the normal control subjects ranged from 2 to 6 gm., 30 per cent of the diabetic patients had values less than 2 gm. and 37 per cent had values exceeding 6 gm. There was good correlation between the clinical types of diabetes and the hepatic responsiveness to insulin. The obese patients with diabetes were mostly in the hepatic insulin-insensitive category while the juvenile type predominated in the insulin-sensitive group. Histologically, there were fatty changes in liver tissue obtained by biopsy in the cases of insulin-insensitivity. Somewhat different results were obtained by Bondy et al., all of whose patients were suffering from diabetic ketosis and none were obese [74]. In such patients the hepatic glucose output was found to be significantly increased. Within fifteen minutes after the first injection of insulin there was a decrease in the arterial blood glucose concentration while the splanchnic glucose balance remained negative until at least forty-five minutes after the administration of insulin. Their conclusion that in the decompensated diabetic patient there is increased hepatic glucose output and a relative



underutilization of carbohydrate does not permit any inference as to which is the primary event. Hepatic glucose production and consequently peripheral carbohydrate utilization was normal in the insulin-controlled diabetic subject [75]. The diabetic animal continues to produce glucose at hyperglycemic levels, while in the normal animal an increase in the blood sugar concentration inhibits hepatic glucose release [76]. This suggested that diabetic hyperglycemia was in part directly due to inability to control hepatic glucose output. Consistent with an increased glucose output from the liver during uncontrolled diabetes are the observations in human subjects and in rats that liver glucose-6-phosphatase activity is increased [77,78]. These values returned toward normal following the administration of insulin but there did not seem to be any correlation between the levels and the clinical types of diabetes [77].

Since it has been assumed that insulin deficiency, either absolute or relative, exists in the diabetic subject, it would seem reasonable that the primary metabolic defect in the diabetic subject occurs in the tissues where insulin acts. The problem of the mechanism of action of insulin is discussed elsewhere in this symposium [2]. Since Gemmill's original observation [79] of a stimulatory effect of insulin on carbohydrate metabolism by the isolated rat diaphragm, there has been ample confirmation of the insulin-responsiveness of muscle [80]. Adipose tissue also responds to insulin stimulation and recently the role of adipose tissue in carbohydrate metabolism has been given more emphasis [81–85]. The insulin-responsiveness of these two tissues is in keeping with the hypothesis that peripheral underutilization of glucose is the primary defect in diabetes mellitus.

In contrast, the question of a direct effect of insulin on hepatic carbohydrate metabolism has not been definitely settled. The evidence for an action of insulin on hepatic metabolism has been summarized recently [86]. DeDuve et al. reported that following hepatectomy there was a considerable reduction in the amount of infused glucose necessary to maintain a constant blood sugar level in the maximally-insulinized dog. The interpretation of these findings as indicative of an action of insulin on hepatic glucose uptake has been questioned [87]. Lang et al. have concluded from similar studies that the liver produces a humoral factor which stimulates peripheral glucose utilization. In the alloxan-

diabetic rat, the abnormalities of hepatic metabolism are corrected very slowly after insulin injections, as compared with the effects observed in muscle [88]. In the depancreatized dog deprived of insulin, the injection of insulin resulted in an abrupt marked increase in the rate of disappearance of plasma glucose and a slow and much smaller decline in its rate of appearance [89]. These results, based on changes in the specific activity of circulating glucose following insulin, confirm the earlier results of Bondy et al. using hepatic vein catheterization [74]. Wall et al. found that the main component of insulin-induced hypoglycemia was an increase in glucose uptake by tissue; of lesser importance was decreased hepatic glucose release [90]. Since these authors did not differentiate between peripheral and hepatic glucose uptake in response to insulin, it is difficult to interpret their results in terms of a direct effect of insulin on the liver. Similar isotope techniques have been used by other workers with variable results [76,91–93]. A simultaneous increase in the rate of glucose removal and complete inhibition of hepatic glucose output following insulin has been observed in normal dogs [76]. In human subjects, both non-diabetic and diabetic, the injection of insulin resulted in inhibition of hepatic glucose output, interpreted as a manifestation of the role of insulin in liver metabolism [91,92]. Ashmore et al. were unable to observe such inhibition of hepatic glucose output in the dog following insulin therapy; in fact, they noted an increase in glucose outflow from the liver in their experiments [93]. Although insulin injected into a peripheral vein and a portal vein both caused the same degree of arterial hypoglycemia, in the former situation there was a significantly greater increase in peripheral utilization of glucose [94].

Although the primary defect in the diabetic patient involves glucose there are also very obvious disturbances in the metabolism of fat and protein [3]. In the diabetic rat the utilization of glucose for fatty acid synthesis was reduced to about 5 per cent of the normal figure [95]. This same defect was observed in liver slices obtained from diabetic rats, whether the substrate was glucose [96,97], acetate [97,98,99] or octonate [98]. The addition of insulin [98] or fructose [97] to the medium did not increase the incorporation of acetate into fatty acids in liver slices from diabetic animals even though such slices could convert fructose to carbon dioxide at a normal rate. Hypophysectomy restored to the

diabetic animal the ability to synthesize fatty acids and suggested the possibility that this defect was not primarily a result of insulin lack [99]. Feeding diabetic rats fructose for four days led to a pronounced increase in the capacity of the liver to convert acetate to fatty acids although there was no change in the defect in glucose utilization [100]. A further elucidation of the problem of reduced fatty acid synthesis in diabetes was provided by the studies of Dituri and his colleagues [101,102]. Fatty acid synthesis from acetate in a particle-free system was found to be stimulated by the addition of citrate but no such effect was observed when another reduced triphosphopyridine nucleotide (TPNH) generating system, glucose-6-phosphate and glucose-6-phosphate dehydrogenase, was added. In the absence of citrate, the process of lipogenesis in the preparation from diabetic rats stopped at  $\beta$ -OH butyrylcoenzyme A. It had previously been shown that TPNH was required in the synthesis of fatty acids for the conversion of crotonyl-CoA to butyryl-CoA [103]. Brady, Mamoon and Stadtman also demonstrated that TPNH was limiting in fatty acid synthesis in the supernatant fraction from pigeon liver and that fatty acid synthesis could be stimulated by the addition of any of several TPNH generating systems [104]. The defect in fatty acid synthesis by supernatant solution obtained from homogenized liver from diabetic animals was abolished by the addition of supernatant fluid from normal rat liver [102]. The active factor in the supernatant was found to be butyryl-CoA. This addition evidently bypassed the TPNH-dependent reductive step, pointing to this reaction as the site of the metabolic defect in fat synthesis in diabetes. Additional supporting evidence for this thesis was provided by the work of Siperstein and Fagan [105,106]. The rate of fatty acid synthesis was found to be dependent on the process of glucose utilization, and specifically on that portion of glucose which was metabolized by way of the hexosemonophosphate pathway. This route of glucose catabolism supplied TPNH, which appeared to be a limiting factor in fatty acid synthesis. These interrelationships between glucose and lipid metabolism are described in greater detail elsewhere [107]. In homogenates made from livers of diabetic rats it was also possible to demonstrate that the defect in lipogenesis was dependent on impaired glucose oxidation, especially via the hexosemonophosphate shunt. The resultant deficiency of TPNH

appeared to be the specific cause of the lipogenic defect in the diabetic liver, since generation of this cofactor either by stimulation of the hexosemonophosphate pathway or by addition of TPN and isocitrate restored fatty acid synthesis [106]. It thus appears that the defect in fatty acid synthesis in the diabetic subject is not a primary one, but is secondary to the impaired utilization of glucose.

The defect in protein synthesis in the diabetic subject has not been fully elucidated. The incorporation of  $S^{35}$ -methionine into muscle protein in the diabetic dog was decreased but could be restored to normal following the administration of insulin [108]. It has also been proposed that insulin is essential to the anabolic effects of growth hormone [109]. However, a less direct effect of insulin was suggested by the studies of Krahll [110]. These showed decreased incorporation of  $C^{14}$ -glycine into various protein fractions in tissue from fasted diabetic rats incubated in a medium without glucose. The addition of insulin to the medium had no effect, whereas addition of glucose and insulin restored the amino acid incorporation to normal. Glutathione synthesis in the liver from the diabetic animal seemed to depend upon the availability of intermediates from carbohydrate metabolism. An *in vitro* effect of insulin on the transport of amino acids into rat diaphragm has been demonstrated, but in these experiments glucose oxidation did not seem to be a necessary prerequisite [111]. A possible explanation for the relationship between protein synthesis and glycolysis was provided by the observation that the addition of TPN stimulates protein synthesis in normal rat liver homogenates [112]. It is quite conceivable that the defect in protein synthesis in the diabetic subject also will turn out to be secondary to decreased carbohydrate utilization.

A defect in the metabolism of two carbon fragments and of the Krebs tricarboxylic acid cycle also has been postulated in diabetes [97, 113-116]. The observations of Siperstein and Fagan [105,106] would appear to explain the second block in carbohydrate utilization in the diabetic liver, acetate conversion to fatty acids, proposed by Chernick et al. [97]. While the oxidation of acetate to carbon dioxide was normal in liver slices from alloxan-diabetic animals [97], it was decreased in diaphragm from such animals [114,117]. Pyruvate oxidation to carbon dioxide also was reduced in muscle from diabetic rats but could be restored to normal by the addi-



tion of insulin [114]. Although insulin was without effect in respect to acetate oxidation, the addition of aconitate and oxaloacetate to the incubation medium increased the production of carbon dioxide from acetate. It was suggested that insulin was involved in the metabolism of pyruvate but that the diminished oxidation of acetate was not a manifestation of insulin deficiency *per se* [114]. Further evidence in the diabetic animal for a block between the metabolism of pyruvate and the Krebs tricarboxylic acid cycle was presented by Frohman et al. [115,116]. These investigators found the concentrations of the acids in the Krebs cycle to be decreased in liver and kidney from diabetic rats whereas the levels of pyruvate and lactate were normal [115]. Insulin injection prior to sacrifice of the animals restored the levels of the Krebs cycle intermediates toward normal, while substitution of fructose for glucose had no effect. Bicarbonate and glucose or fructose administration to the diabetic animal seemed to restore to normal the incorporation of  $C^{14}$ -acetate in the Krebs cycle intermediates without the concomitant injection of insulin. The bicarbonate effect was attributed to correction of the decreased pH in the uncontrolled diabetic subject or to stimulation of the formation of oxaloacetate from pyruvate and carbon dioxide [116,118]. In human diabetic subjects the blood level of  $\alpha$ -ketoglutarate has been reported to be normal, and no differences were found in the patients with various clinical types of diabetes [17]. A defect in acetylation in the alloxan-diabetic rat has been described [119], but contrary results have been reported in human diabetic subjects [120]. Despite the fact that the postulated block in acetate condensation with oxaloacetate in the diabetic subject was not believed to be insulin-dependent [114], it has not been possible to reduce the ketosis either in diabetic animals or in diabetic man by the administration of succinic acid [121-123] or malic acid [123]. Although the defect in diabetes has been definitely shown to be secondary to the impaired glucose metabolism only in respect to acetate conversion to fatty acids, it would seem likely that the other abnormalities in the metabolism of acetate and Krebs cycle intermediates also are secondary to alteration in glucose oxidation.

#### CONCLUDING REMARKS

It should be obvious that the metabolic abnormality which occurs in the human diabetic

subject is quite complex and incompletely elucidated. Although studies in diabetic animals have produced much information pertinent to the problem as it is encountered in man, there does not appear to be any real animal counterpart to the various clinical types of diabetes. The different types of diabetes are included together under the present definition of the disease, but to date proof is lacking that the disorder in all diabetic patients reflects either the same etiologic agent or the same altered biochemical defect. Judged both by assay of insulin-like activity in the plasma and the amount of extractable insulin in the pancreas, there is evidence for at least two separate types of diabetes although these distinctions are not always absolute. For the most part, there appears to be good correlation between these parameters and the types of diabetes defined clinically. Despite some evidence to the contrary, the juvenile diabetic subject appears to be suffering from a primary failure of the pancreas to produce insulin while the mild, obese, adult diabetic patient has evidence of some pancreatic function. In this latter group of patients, the reason for the apparent deficiency of insulin is not clear. Suggested possibilities have included an excessive demand for insulin secondary to the increased conversion of glucose to fat as part of the obesity, and the failure of hepatic glucose production to respond to insulin. Again the experimental evidence is somewhat conflicting and does not conclusively support either hypothesis. The question of a direct insulin effect on hepatic carbohydrate metabolism is still controversial, whereas the responsiveness of both muscle and adipose tissue to this hormone has been consistently and repeatedly demonstrated. There is both underutilization and overproduction of glucose in uncontrolled diabetes, but it has been difficult to determine which is the primary event or whether both factors are equally important in producing diabetic hyperglycemia. Most of the evidence points to the fact that the diabetic subject at high blood sugar levels can utilize as much glucose as the normal subject at physiologic blood sugar concentrations. Consequently overproduction of glucose might be construed as a compensatory mechanism. Glucose oxidation to carbon dioxide might be much more impaired in the juvenile diabetic subject than in the obese diabetic subject but there is a paucity of human experiments pertinent to this point. The impaired ability of the diabetic subject to synthesize fatty acids does

not appear to be a primary metabolic defect but rather to be dependent upon the decreased availability of reduced pyridine nucleotides consequent to the reduction in glucose utilization. The defects in protein synthesis and the Krebs tricarboxylic acid cycle probably are also secondary to the primary failure in glucose utilization although the experimental evidence is not as conclusive as in the case of fat synthesis. In short, much has been learned in the past concerning the nature of the metabolic defect(s) in diabetes, but much still remains for future investigation.

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# The Action of Insulin on the Transport of Glucose Through the Cell Membrane\*

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THE stimulation of glucose uptake by muscle and certain other tissues is generally recognized as one of the most characteristic and important actions of insulin. This acceleration is without doubt the principal cause of the fall in blood sugar following administration of the hormone. The absence of this effect accounts in large part for the abnormal elevation of the blood sugar in certain types of diabetes. It therefore seems clear that stimulation of glucose uptake is an appropriate phenomenon for investigation of the locus of insulin action.

For the purpose of the present discussion, glucose uptake in muscle can be broken down into three steps. The first is the passage of glucose from the blood plasma to the cell; the second is the transport of the sugar through the cell membrane; and third is the metabolism, or utilization, of glucose inside the cell. Since these steps follow one another in sequence, the over-all rate of uptake will be influenced by the resistance offered to the flow of sugar by each step individually. The predominant control, however, will be exerted by the step with the highest resistance. Since insulin may cause a several-fold increase in uptake, it is reasonable to assume that this "rate-limiting" step has been accelerated. Thus it is possible to restate the problem of where insulin acts by asking which is the rate-limiting step for glucose uptake in the muscle tissue.

With these considerations in mind, each of the steps will be examined with regard to the following questions: what are the physiological and biochemical processes involved; to what extent does the step in question control the over-all rate of glucose uptake; and what is the evidence for acceleration by insulin?

## *The Passage of Glucose from the Blood Plasma*

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to the Cells. The extracellular movement of glucose from the plasma to the cells in muscle has not been studied in detail. The process is thought to be largely a matter of physical diffusion through capillary "pores" and across the interstitial space. The process is rapid, as shown by the finding that labelled glucose, injected intravenously into eviscerated-nephrectomized rabbits, reached a complete distribution in the extracellular water within twenty minutes, when the first measurements were made [1]. Of possibly more significance is the observation [2] that the concentration of extracellular glucose in muscle is close to that in the blood plasma in the absence of insulin. This must mean that the rate of glucose entry into the interstitial water is quite fast relative to the rate of its removal by the cells. In isolated muscle preparations, it has been found that the extracellular distribution of small molecules which do not penetrate the cells is rapid [3]. For example, in the isolated, perfused rat heart, the half-time for the equilibration of sorbitol between the blood plasma and interstitial water is less than one minute [4]. Since this substance is very similar to glucose in physical properties it would be expected to diffuse at the same rate.

It would appear from these considerations that no major barrier exists for glucose penetration until the cell wall is reached. It would appear improbable, therefore, that any major effect of insulin would be found on this step in glucose uptake. This conclusion is supported experimentally by the finding that insulin causes a marked increase in uptake by the isolated rat diaphragm [5] and the lens of the eye [6] where effects on capillary circulation and permeability can play no role. The hormone, furthermore, does not affect the process of diffusion *per se* in the extracellular

water since the distribution rate of small molecules such as thiosulfate [3] and sorbitol [4] is not influenced by either alloxan diabetes or insulin. The possibility, however, that insulin may have a minor effect on the rate at which glucose reaches the cell has not been excluded. It is apparent that this rate must be greatly increased when the removal of glucose from the interstitial water by the cells is increased with insulin. Under these conditions, extracellular transfer could impose some limitation on the uptake process and a hormone effect which accelerated transport through the capillary membrane, for example, could have some physiological importance.

*The Membrane Transport of Glucose.* Levine and associates [7-10] first suggested that transport through the cell membrane might be the rate-limiting step for glucose uptake and the point of insulin action. This proposal has focused attention on the question of how glucose enters the cell in muscle tissue and has stimulated a number of investigations of this problem.

*The concept of a membrane carrier system:* The permeability of membranes to most physiological substances is very poorly understood, despite the obvious great importance of this process to the cell economy. With regard to glucose and other monosaccharides, the best and most extensive permeability studies have been carried out in the erythrocyte. It may be helpful, therefore, to outline briefly the concept of transport which has been developed for this cell before proceeding to the muscle cell. As will be shown, the basic mechanism for permeability to sugar is similar in the two cell types; muscle, however, appears to have some superimposed mechanism for hormonal control.

The red cell has particularly suitable properties for transport studies. It is a free cell which is simple in structure and metabolic pattern. In the erythrocyte of man and other species it is relatively easy to separate glucose metabolism from membrane permeability and to measure the latter by both rapid optical methods and direct chemical analysis.

From the work of Wilbrandt [11-14], LeFevre [15-19], Widdas [20-22], Rosenberg [23,24] and others [26-28] there has emerged the concept that the transport of a number of monosaccharides, including glucose, occurs by means of a specialized "carrier system" which in its simplest form can be represented as shown in Figure 1.

Glucose on the outside,  $G_o$ , is postulated to complex with a substance,  $X$ , in the membrane.

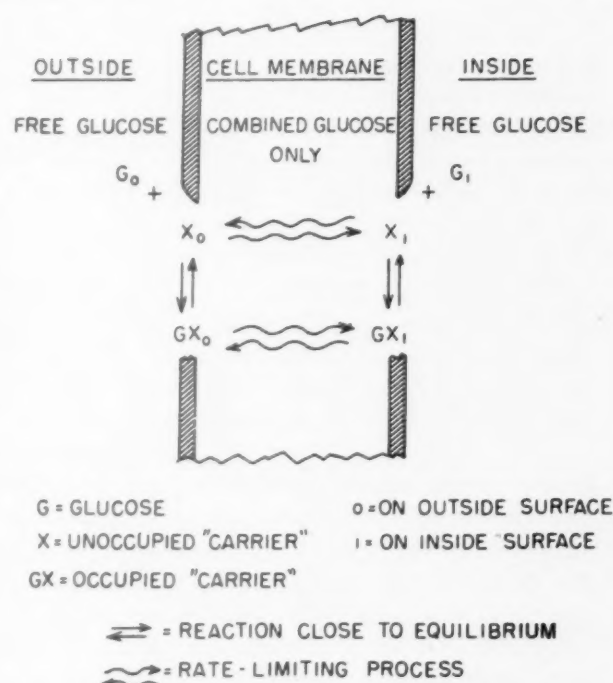


FIG. 1. A model for glucose transport by a membrane carrier system. The model is described in the text. Various proposals for carrier systems have been discussed in some detail by Rosenberg [14,23]. The transport equations applying to the version indicated here have been presented elsewhere [27].

The complex,  $GX$ , then passes across the membrane to dissociate and release free sugar into the cell  $G_i$ . The process is reversible. The principle lines of evidence in favor of such a scheme are as follows:

(1) When the permeability of two closely related sugars such as D- and L-galactose is studied, it is found that the former enters the cell rapidly whereas the latter is excluded [29]. Since these two sugars are very similar in size and solubility, permeation by simple diffusion through holes or "pores" in the membrane appears most unlikely.

(2) When the entrance of a sugar such as glucose is measured as a function of the extracellular concentration, it is found [11,15,21,22] that the transport rate increases rapidly at low concentrations but approaches a plateau at higher concentrations. (Fig. 2.) The curve does not fit with the kinetics of simple diffusion but can be described in terms of the familiar Michaelis-Menten equation for an enzyme-catalyzed reaction. The curve can be most readily interpreted as being due to approaching saturation of a combining site necessary for transport in the membrane. When other sugars are tested, a family of curves is obtained, some of

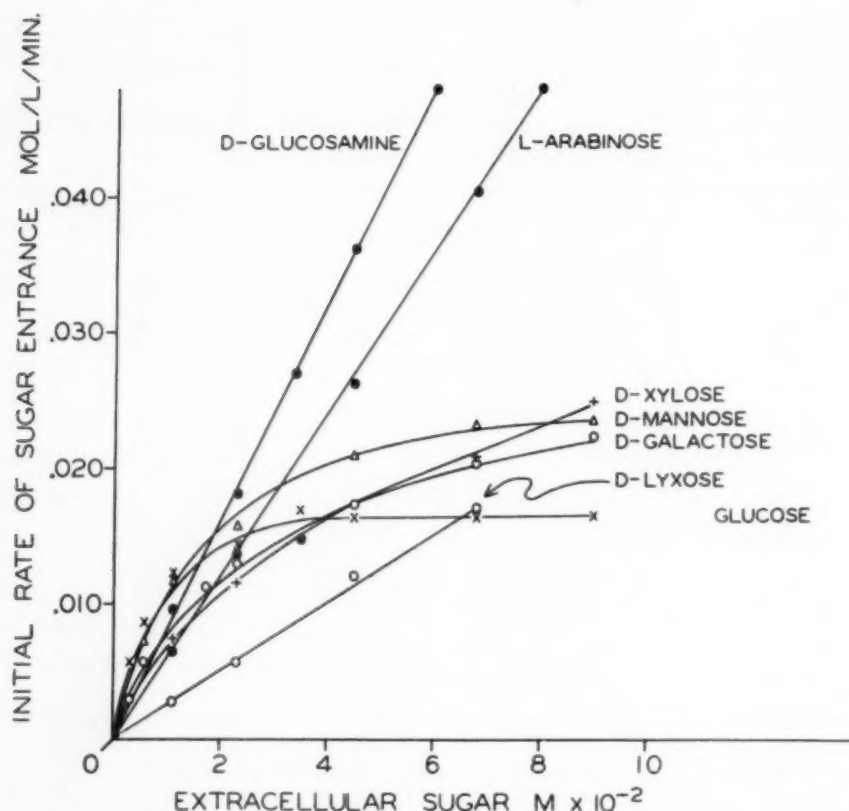


FIG. 2. The relationship of transport rate to the concentration of monosaccharide. Experiments were carried out in the human erythrocyte at 19°C. Estimates of the initial rates of sugar transport into the cell were made by a modification of the optical technic for determining osmotic swelling originally described by Orskov [26]. The curve for 3-O-methyl glucose (not shown) is superimposable on that for glucose. The transport of all these sugars except D-glucosamine is competitively inhibited by glucose. (REINWEIN, D. and PARK, C. R. Unpublished data.)

which are shown in Figure 2. It has been found that 3-O-methyl glucose, D-galactose and D-xylose, in this order, have relatively high affinities for the combining site, leading to rapid transport at low concentration but early saturation at higher concentrations [16,19,21,28]. L-Arabinose, a number of other common pentoses, and D-fructose (not shown) have such a low affinity that no saturation can be demonstrated in the concentration range that can be studied.

(3) When sugars, including the hexoses and pentoses mentioned, are present in pairs, there is competitive inhibition of transport [16,21,29]. This suggests strongly that these sugars enter by a common transport system. Furthermore, this competition under certain conditions can lead to movement of one of the sugars against a concentration gradient [24,27], an observation which provides perhaps the strongest evidence for a carrier mechanism, as will be discussed subsequently.

(4) A number of chemical agents, such as parachloromercuribenzoate in low concentration, inhibit the transport system [15]. This points to the involvement of specific chemical groupings in the membrane, probably sulfhydryl groups in this instance. Phlorhizin is a potent competitive inhibitor of the transport process [11,16,30].

(5) The temperature coefficient for transport is in the range of two to threefold per 10 degree interval, which is much higher than expected for physical diffusion although it does not conclusively exclude such a process [15,16,28].

From the foregoing observations it would appear to be established beyond reasonable doubt that a mechanism, analogous in many respects to an enzyme system, is located in the erythrocyte membrane and has the function of facilitating the selective uptake of a variety of monosaccharides. The process does not lead to concentration of sugar against a gradient except



in the special competitive situation already mentioned. The energy requirement is met by the kinetic (thermal) energy of the molecules in solution and not by metabolism within the cell. Thus the process can be distinguished on the one hand from "active transport," which involves movement of a substance against an energy gradient, as seen, for example, in the kidney, gastrointestinal tract and bacterial systems. On the other hand, it is distinct from simple physical diffusion in which no specific interaction occurs between the membrane and the transported substance. The use of the term "transport" may perhaps be justified by these considerations.

*Transport process in muscle:* The studies of Levine and associates [7-10] showed that a number of hexose and pentose analogs of glucose were largely retained in the extracellular water of eviscerated-nephrectomized dogs, but entered the intracellular water rapidly following administration of insulin. It could be inferred from the magnitude of the distribution changes that penetration of muscle cells was involved. This effect appeared to be on permeability rather than on intracellular utilization since several of the analogs were very slowly phosphorylated, if at all. Wick et al. [31,32] confirmed Levine's observations in principle and added the important observations regarding the permeability process that sorbitol, which is closely related to the sugars in physical properties, did not penetrate the cells at all, and that the penetration of galactose, a slowly metabolized sugar in the eviscerated rabbit, was inhibited by glucose. Ross [34], studying penetration from the blood into the aqueous humor, and Park and associates [2,27,33,35], working directly with muscle in eviscerated rats, obtained evidence that permeability for glucose itself was accelerated by insulin, and that phosphorylation was not necessarily involved.

These experiments with whole or eviscerated animals established the importance of the permeability process and the effect of insulin but did not allow sufficient experimental control for a detailed examination of these phenomena. For this purpose *in vitro* preparations are superior, among which the most useful appear to be the so-called "intact" rat diaphragm [3] and the isolated perfused rat heart [36,37]. In both preparations the sugar must pass through the membrane to enter the cell since none of the muscle fibers are transected. The diaphragm, which is excised with a protecting ring of rib

cage, is simpler to handle but lacks important advantages of the heart preparation. These are (1) the diffusion of sugars and other substances to the cell wall is facilitated, since the medium is carried through the tissue by the normal vascular channels; (2) transport and utilization of sugar can be measured simultaneously; and (3) the mechanical activity of the muscle and the perfusion pressure provide convenient indices of the physiological condition of the tissue. It has become quite clear that these technical considerations are of much importance and may account for some of the discrepant findings among various laboratories.

In the isolated, perfused heart from normal rats, 3-O-methyl glucose (3OMG) penetrates the cells [39] even in the absence of insulin. This is shown by the fact that the distribution, or "space," exceeds that of sorbitol, which is used to estimate the extracellular volume [36-38]. In recent experiments [37] we have attempted to determine the maximum possible distribution such a sugar can reach inside the cell and find that only 70 to 80 per cent of the intracellular water is involved. This suggests that some intracellular structures are relatively impermeable. Severe anaerobiosis, metabolic poisons or insulin itself do not cause any gross change in the final distribution. This argues strongly that no "active transport" against concentration gradients occurs under ordinary conditions in this cell.

The relationship of sugar structure and concentration to permeability in muscle is similar to that observed in the erythrocyte. In the case of 3-O-methyl glucose, penetration is relatively rapid at a low external concentration and does not increase proportionately as the external concentration is raised [37]. In other words, 3OMG behaves as if it had a high affinity for a combining site in a transport system, leading to rapid rates at low concentration, but early saturation at higher concentrations. The behavior of D-xylose is similar, whereas that of L-arabinose indicates an appreciably lower affinity.

Differences in affinity have also been shown by inhibition studies [36,39] some examples of which are shown in Table 1. Glucose, galactose and 3OMG depress the transport of each other and of xylose,\* but inhibit much more strongly the

\* Kipnis and Cori [3] failed to find glucose inhibition of xylose penetration in the rat diaphragm. This may have been due to the use of a cut diaphragm preparation and measurements made too close to the final equilibrium distribution of the pentose.

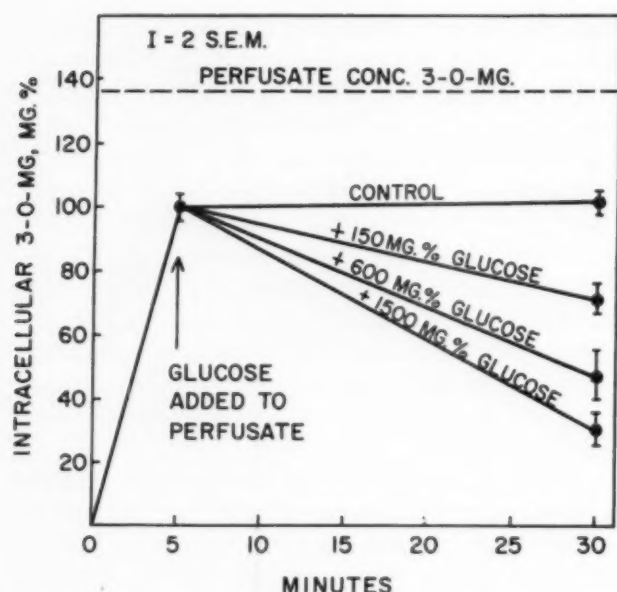


FIG. 3. Extrusion of 3-O-methyl glucose against a concentration gradient due to competition with glucose for the transport system. The experiment is outlined in the text and details will be published elsewhere [4].

penetration of L-arabinose. On the other hand, L-arabinose depresses glucose entry only when the pentose to hexose molecular ratio is high. The order of affinity among these sugars appears to be the same as found in the erythrocyte. Furthermore, phlorhizin is also a potent inhibitor of sugar transport in the muscle [38]. (Table I.)

As in the erythrocyte system, transport of a sugar against a concentration gradient can be induced [39] in a special situation involving transport competition, a demonstration which perhaps provides the strongest argument for a "carrier" mechanism in the membrane. Such an experiment is illustrated in Figure 3. The muscle is perfused with 3OMG until a near maximum accumulation of sugar inside the cell has been reached. Under these conditions the rates of entrance and exit from the cell must be nearly equal. When glucose is added to the external medium at this point, 3OMG is observed to leave the cell, although it is moving into a region of higher concentration. This "against the gradient" transport appears to provide conclusive evidence against simple physical diffusion, since such a process could only proceed downhill in a concentration gradient (see discussion by Rosenberg [40]). The phenomenon also appears to be incompatible with the "polar creep" hypothesis of permeability discussed by Stein and Danielli [47] and Bowyer [22,25], but finds ready inter-

TABLE I  
COMPETITION FOR TRANSPORT BETWEEN SUGARS  
AND THE EFFECTS OF INSULIN AND PHLORHIDZIN  
IN THE PERFUSED, ISOLATED RAT HEART

Additions to Medium	Sugar Utilized, (mg./gm./hr. $\pm$ S. E.)	Intracellular Free Sugar, % of Perfusate Concentration $\pm$ S. E.
Perfusion with L-arabinose, 200 mg. %, 10 minutes		
None	0	43.4 $\pm$ 4.0
Glucose (240 mg. %)	0	14.8 $\pm$ 2.0
3OMG (260 mg. %)	0	13.0 $\pm$ 3.0
Phlorhizin ( $3 \times 10^{-3}$ M)	0	0.0 $\pm$ 2.0
Insulin	0	68.8 $\pm$ 2.0
Perfusion with 3-O-methyl glucose, 600 mg. %, 15 minutes		
Insulin	0	62.4 $\pm$ 2.7
Insulin plus glucose (600 mg. %)	0	38.8 $\pm$ 2.5
Perfusion with glucose, 150 mg. %, 15 minutes		
None	4.5 $\pm$ 0.5	0
Insulin	9.6 $\pm$ 0.4	32 $\pm$ 3
Insulin + L-arabinose (1500 mg. %)	7.4 $\pm$ 0.6	17 $\pm$ 3
Insulin + 3OMG (600 mg. %)	5.6 $\pm$ 0.6	0

NOTE: Hearts from normal, fasted rats were perfused at 37° by recirculation through the coronary system of 7 to 10 ml. of bicarbonate-buffered medium. Details will be presented elsewhere [4]. The utilization and intracellular concentration columns refer only to the sugar originally present in the medium and not to the sugar added. It will be noted in the bottom panel that insulin accelerates entrance of glucose sufficiently to accumulate free sugar inside the cell. L-Arabinose and 3-O-methyl glucose (3OMG) reduce the level of intracellular free glucose. This shows that their inhibitory effect is on the transport process, since inhibition at the level of intracellular metabolism would cause a further rise of free sugar inside the cell.

pretation in a carrier hypothesis as outlined in Figure 1. In these terms, the explanation is as follows: glucose competes effectively at the external surface of the membrane and reduces the entry of 3OMG. Inside the cell, however, the glucose which has entered is metabolized and therefore does not compete with 3OMG exit. The latter flux, therefore, temporarily exceeds the rate of entrance until the 3OMG concentration inside the cell falls low enough to establish a new equilibrium of transport rates. Energy for the concentration process is derived originally from the inward movement of glucose down its concentration gradient. This establishes in turn an outwardly directed concentration gradient of carrier in the membrane itself which is available for 3OMG and provides the immediate energy for extrusion of the sugar. Goldstein [42] has recently carried out an analogous experiment using an insulinized, eviscerated-nephrectomized dog in which xylose was injected with sufficient lapse of time to permit accumulation of intra-

cellular sugar and the attainment of a stable blood pentose concentration. The injection of glucose at this point led to a substantial rise in the blood xylose level, an indication that hexose-pentose competition and the transport kinetics described are not special properties of the heart but presumably apply to muscle tissues in general.

From the foregoing discussion, the similarities of monosaccharide transport in the erythrocyte and muscle are apparent. The system appears in many respects analogous to a reversible enzymatic reaction which leads to translocation of substrate rather than a transformation. In regard to the problem of physical translocation, the thinness of the cell membrane, which has been estimated to consist of only a few layers of lipid and/or protein, should be kept in mind. A "carrier-sugar complex" could be imagined to oscillate only by virtue of thermal agitation between the membrane surfaces. An attractive speculation regarding the nature of a sugar carrier is based on the concept that the high lipid content of cell membranes imposes a barrier to penetration by lipid-insoluble substances such as the sugars. If a sugar, however, were to combine with a suitably large lipid membrane component the complex would still retain enough solubility to pass this barrier.

*The effect of insulin on sugar transport:* Since the pioneer experiments of Levine and associates, an acceleration of sugar transport by insulin has now been shown by many investigators using non-metabolizable analogs of glucose. The phenomenon has been demonstrated in the dog [7-9], rabbit [32], cat [43b], rat [35,43a] and man [44], and in numerous isolated tissue preparations [3,6,33,36,45,46]. An illustrative example using L-arabinose in the isolated heart preparation has been included in Table 1. It can also be seen that insulin accelerates the accumulation not only of the non-metabolizable sugar but also of glucose. Competition for transport in the presence of the hormone is also clearly demonstrated as shown by the data with 3-O-methyl glucose.

The effect of insulin on transport is particularly well observed by an improved technic which makes use of the fact that transport is a reversible process, sensitive to hormonal influences in the outward as well as inward direction. In this method, the isolated heart is first perfused with a high concentration of L-arabinose which accumulates inside the cell. The perfusion

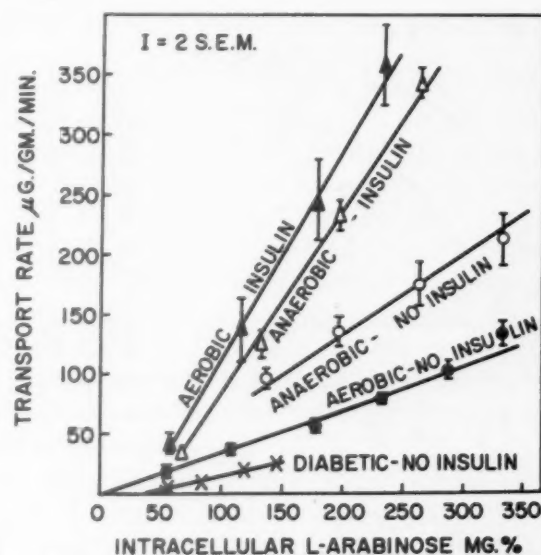


FIG. 4. Transport of L-arabinose out of the cell as a function of the intracellular pentose concentration. The experimental procedure is outlined in the text and details will be published elsewhere [4]. Diabetes was induced by alloxan forty-eight hours previously. Insulin was present, where indicated, in a concentration of 3  $\mu$ g./ml.

is then shifted to sugar-free medium which is collected over intervals of a minute or so as it leaves the heart. Five minutes of this washing removes 98 per cent or more of extracellular pentose, as judged by the wash-out of an extracellular indicator substance such as sorbitol-1- $C^{14}$ . The measurement of pentose in the perfusate subsequent to this time then provides a measure of L-arabinose efflux from the cell. By a simple calculation it is possible to obtain a curve of outward transport as a function of intracellular concentration. The particular sensitivity of this method derives from the fact that transport under these conditions is essentially unidirectional, since the continuous removal of extracellular sugar keeps transport back into the cell at a minimum. Figure 4 shows a few typical results. Insulin causes a three to fourfold increase in rate over the normal control. Alloxan diabetes induces a 50 per cent depression of transport, which can be increased about sixfold with insulin *in vitro* to obtain the same high level reached in the insulin treated, normal heart. These values illustrate the very broad range of rates under hormonal control. In regard to the diabetic muscle, the depressed transport cannot be ascribed simply to insulin deficiency, since removal of the adrenal or pituitary restores pentose efflux to normal or above [47]. The anaerobic effect [48] shown in Figure 4 will be



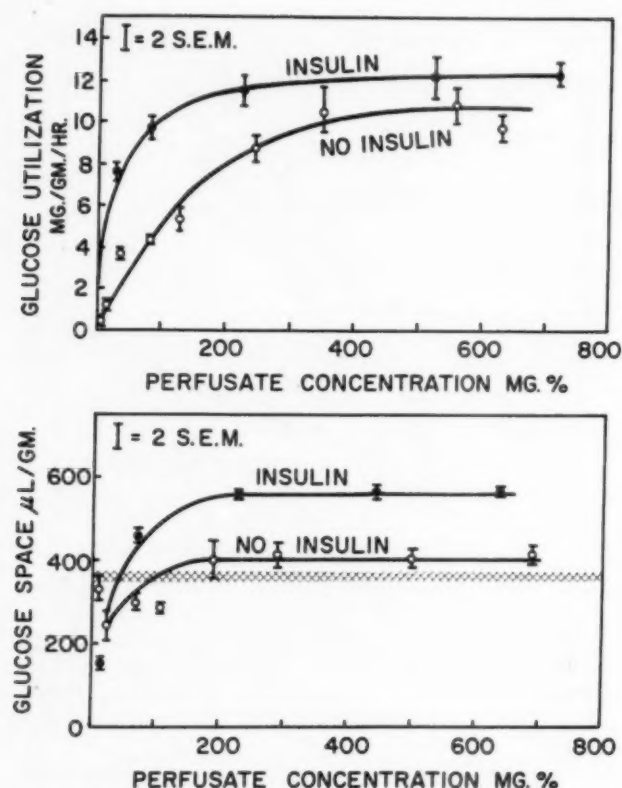


FIG. 5. The effect of insulin on the utilization and intracellular accumulation of glucose at various external glucose concentrations. The experiments were carried out using the isolated, perfused rat heart. The procedure is outlined in the text and details will be published elsewhere [37].

discussed subsequently, but it may be noted here that insulin retains its effectiveness under these conditions.

The time course of the insulin effect can also be well observed by the outward transport technic [4]. The first acceleration is noticed within about one minute and reaches a maximum in five to eight minutes. If some time is allowed for the extracellular diffusion of the hormone to the cell it would appear that the response begins almost immediately. These time relationships, together with the consideration that no "pores" exist in the membrane for much smaller molecules, suggest that the first point of hormone interaction is at the surface rather than within the cell. In this connection, Stadie and associates [49,50] have presented evidence for a binding of insulin by muscle tissue.

The competition experiments discussed earlier make it seem very probable that glucose is transported by the same mechanism as the non-metabolizable sugars and provide much justification for extending observations with glucose

analogues to the transport of glucose itself.\* Only studies with the latter sugar, however, can provide an answer to the question raised at the outset, namely, is transport the step which is predominantly rate-limiting for glucose uptake by the muscle. A number of experiments using intact or eviscerated rats [2,35,27] and isolated muscle preparations [33,36] have suggested an affirmative answer to this question. A recent series of experiments, however, provide a more definitive answer [37] and illustrates the reasoning involved.

In this study, hearts from normal rats were perfused at various concentrations of glucose with and without insulin. At the end of the run, estimations were made of the uptake of sugar from the medium and the amount of free glucose inside the muscle cells. The uptake measurement provided a value for glucose utilization, or phosphorylation, after a correction was made for any change in the free sugar content of the tissue. The estimation of intracellular free sugar provided an indication of the balance between the rates of entrance and phosphorylation, as will become clear from what follows.

The analysis of the results, shown in Figure 5, may be begun by inspection of the values obtained when insulin was present. The curve for glucose utilization rose rapidly and levelled out when the external concentration was about 300 mg. per cent. Corresponding to this plateau, there was uniformly a large accumulation of free glucose within the cell (bottom panel). The interpretation was as follows: Entrance, or transport of glucose into the cell, rose rapidly with the increase in external concentration and, at about 300 mg. per cent, became so rapid that the capacity for intracellular phosphorylation was exceeded and free sugar piled up inside the cell. Under these conditions, it is clear that intracellular phosphorylation was rate-limiting to glucose uptake since the accumulation of intracellular free sugar would in turn reduce

\* Levine and associates suggested originally [10] that sugars having the same configuration as glucose in the first three carbon atoms were "insulin-responsive." This point has been questioned by others [33,43] and also by Levine himself [62]. In my opinion, the configuration of the sugar has importance only with regard to the specificity requirement of the transport process itself and probably conforms to that suggested by LeFevre [19] for the erythrocyte. Thus all sugars which can be transported by the glucose system will be insulin-responsive. If the basal rate, however, is very slow even a very large acceleratory effect of insulin may not become detectable by present methods of testing.

entrance from the medium. This can be now compared to the results obtained in the absence of insulin. Here the utilization of sugar also rose with increasing levels of extracellular glucose, but the rise was slower and the plateau was not approached closely until the external concentration reached about 600 mg. per cent. Virtually no free intracellular sugar was found over the whole range of external concentration up to the highest values. These findings were explained as follows: Transport of sugar into the cell was now slower than the capacity for phosphorylation within the cell; all glucose entering could be immediately metabolized and no free sugar accumulated. Thus the rates of uptake and utilization were equal under these conditions and were limited by the transport process. When the insulin and no insulin results are now compared, it is seen that glucose utilization, which was equivalent to uptake from the medium except for the relatively small changes in free sugar, was markedly stimulated by insulin *over the range of concentration where transport was the limiting step*. At the highest concentration, where transport was becoming fast enough to approach saturation of the phosphorylation capacity, the insulin effect was much reduced. The latter observation suggests strongly that the hormone has no direct effect on intracellular glucose utilization but stimulates this process only secondarily by acceleration of membrane transport.

*The Intracellular Utilization of Glucose.* In 1949, Cori [63] suggested that the hexokinase reaction was the rate-limiting step for glucose uptake by muscle and was accelerated by insulin. This reaction, in which glucose and adenosine triphosphate react to yield glucose-6-phosphate, was thought to be the first enzymatic step and one through which all the utilization of glucose was funnelled. The reaction involves a considerable release of free energy and is therefore practically irreversible, a consideration of importance since it appeared to exclude as rate-limiting the subsequent steps which might pile up glucose-6-phosphate. Evidence was obtained that hexokinase activity was not limited by the supply of adenosine triphosphate [51]. A hormone effect on the hexokinase system, furthermore, appeared to fit with studies [52,53] with muscle extracts in which an acceleration of glucose phosphorylation with insulin was observed, although not with the desired reproducibility. Subsequent work, however, has made acceptance of the "hexokinase theory" difficult. Among other

considerations, Weil-Malherbe and Bone [54] and Crane and Sols [55] have shown that the hexokinase reaction is in fact inhibited by its product, glucose-6-phosphate. It has been suggested [56], for example, that epinephrine may depress glucose uptake in muscle through the build-up of inhibitory levels of glucose-6-phosphate derived from the rapid breakdown of glycogen. Despite much effort, the effect of insulin in muscle extracts has not been confirmed satisfactorily [57,58]. Probably the most important single objection, however, to the hexokinase theory has been the growing awareness of membrane transport as a specific, insulin-sensitive step which precedes phosphorylation in the uptake process.

While a direct action of insulin on intracellular phosphorylation appears to be unlikely, this process may become rate-limiting for uptake as a result of other influences of a hormonal nature. The epinephrine effect has already been cited, and Kipnis and associates [59] have recently presented evidence for a metabolic block at the phosphorylation level with prolonged fasting. While transport appears to be the first metabolic block for glucose uptake in diabetic muscle [35], Morgan and associates [48] have shown that intracellular utilization of glucose is also depressed. This inhibition greatly reduces the immediate response to insulin.

#### CONCLUDING REMARKS

A central problem for the future appears to be the clarification of the transport process and insulin effect at the level of specific chemical reactions about which nothing is at present known. It is obvious that a complex sequence of reactions may be contained in the events that can now be described. In this connection, Randle and Smith [46] have recently published an interesting proposal which may offer a new point of attack. In experiments with the rat diaphragm, they observed that anaerobiosis or metabolic poisons which interfere with the production of phosphate bond energy cause an acceleration of sugar transport. This has been confirmed in the isolated heart preparation [48] and representative experiments have been shown in Figure 4. The effect is not apparently due to a non-specific breakdown of the cell membrane, since sorbitol, for example, does not enter the cell under these conditions [48]. In addition to the effect on transport, anaerobiosis also greatly

stimulates intracellular glucose phosphorylation [48]. The phenomenon is of great interest and may explain in part the increase in glucose utilization with muscular exercise, which always involves some degree of anaerobiosis. Randle and Smith advance the novel concept that metabolic energy may be necessary to *restrain* glucose entry into muscle and that insulin could be conceived to be an agent which breaks specifically the link between the energy source and the membrane.

Although the present article has been focused on the relationship of insulin to glucose transport in muscle, it is not intended to suggest that this is the only effect of the hormone, particularly in view of the recent evidence [60,61] pointing to insulin effects on amino acid uptake and incorporation into protein in the apparent absence of glucose. Furthermore, a number of observations with regard to the metabolism of fats are not at present interpretable in terms of sugar transport [50]. The final solution to the problem of insulin action must of course provide some common denominator for these various effects which cannot be related in any obvious manner at the present moment.

#### SUMMARY

The properties of the process for the transport of monosaccharides through the cell membrane in the erythrocyte and muscle are reviewed. Transport appears to be distinct from simple physical diffusion and involves an interaction of the sugar with a specific site on the cell membrane. The process is reversible and free sugar is liberated at either surface of the membrane. There is no evidence that sugars are moved against concentration gradients under ordinary circumstances and no metabolic energy is required. In fact, a reduction in the level of phosphate bond energy in muscle leads to more rapid transport of glucose and other sugars. Competition for transport can be shown among the hexoses and pentoses which have been tested, indicating that all these sugars cross the membrane by a common system. Transport antecedes and is distinct from phosphorylation by the hexokinase system and may be regarded as the first step in glucose metabolism. A sugar carrier system in the membrane provides an attractive working concept of the function of the transport process.

A characteristic action of insulin is the stimulation of glucose uptake in muscle. This effect must

be due to acceleration of whatever step is rate-limiting in the uptake process. Present evidence indicates that membrane transport is predominantly rate-limiting in the absence of insulin and that the hormone accelerates this step. Increased entrance of glucose provides more substrate for the hexokinase system and results in a rise in the metabolism of the sugar. The effect of insulin and other hormonal factors on transport can be particularly well observed by techniques employing non-metabolizable analogs of glucose. Such studies show that a very broad range of transport rates is under hormonal control. The acceleratory action of insulin is very rapid and probably occurs through a primary interaction at the cell surface. The elucidation, however, of transport and the insulin effect on the level of specific molecular interactions remains a problem for the future.

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# Inter-relationships of Glucose and Lipid Metabolism\*

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OVER the course of the past five years a considerable advance has been made in our understanding of how glucose may influence a number of phases of lipid metabolism. The elucidation of this problem has been greatly aided, on the one hand by studies on the details of glucose breakdown, and on the other by a number of fundamental advances in our knowledge of the biochemical mechanisms of lipogenesis and of ketogenesis. It should be emphasized at the outset of this review, however, that there remain today numerous unanswered questions in this field, especially in regard to the relationship between glycolysis and the synthesis of ketone bodies and of cholesterol.

There can be little doubt that the most important advance in this field stems from the realization that glucose can be metabolized in most cells of the body by at least two different pathways. This conclusion was first reached independently by Lipmann [1] and by Dickens [2] in 1936; however, it has only been during the past few years that the details of the biochemical reactions involved in the so-called pentose phosphate pathway of glucose breakdown have been fully described [3,4]. Furthermore, only recently have methods been developed for determining the relative quantitative significance of the two major pathways of glucose catabolism.

## GLUCOSE BREAKDOWN

*Embden-Meyerhof Pathway.* The more familiar of the two routes of glycolysis† is the Embden-Meyerhof pathway. It is operative in almost every cell of the body and, with two or three exceptions to be discussed later, is no doubt the major route by which glucose is oxidized in

animal tissues [5]. The biochemical steps involved in the metabolism of glucose over the Embden-Meyerhof pathway are shown in detail in Figure 1. Glucose derived either from the diet or by synthesis in the liver must be phosphorylated by adenosine triphosphate (ATP) to form glucose-6-phosphate before it can be further metabolized. Insulin, in some manner, plays its role in cellular metabolism by increasing the rate of this reaction. At the present time it seems likely that insulin accomplishes this end by increasing the permeability of muscle [6] and probably of adipose tissue [7] to glucose, although in the liver glucose is freely permeable and insulin may specifically enhance the action of glucokinase on the intracellular glucose [8].

Following phosphorylation, glucose to be metabolized over the Embden-Meyerhof pathway is converted to fructose-6-phosphate through the action of the enzyme, glucose phosphate isomerase. A second phosphate is then added to the fructose phosphate and the resulting fructose-1,6-diphosphate is split into two triose phosphates. As shown in Figure 1, the glyceraldehyde-phosphate, which is ultimately produced, undergoes a series of reactions which eventuates in the formation of pyruvate. During this process one molecule of reduced diphosphopyridine nucleotide (DPNH) (Reaction 5, Fig. 1) and two of ATP (Reactions 6 and 9, Fig. 1) are synthesized for each molecule of glyceraldehyde phosphate converted to pyruvate. Both of these co-factors, as will be discussed later, play significant roles in the synthesis of fatty acids. In the complete breakdown of glucose, pyruvate is converted to acetyl CoA with the loss of one molecule of CO<sub>2</sub> and the formation of DPNH (Reaction 11). In the presence of oxygen, the remaining carbons of glucose are then oxidized to carbon dioxide in the Krebs cycle,

† Glycolysis is used in this paper to connote either pathway of glucose breakdown.

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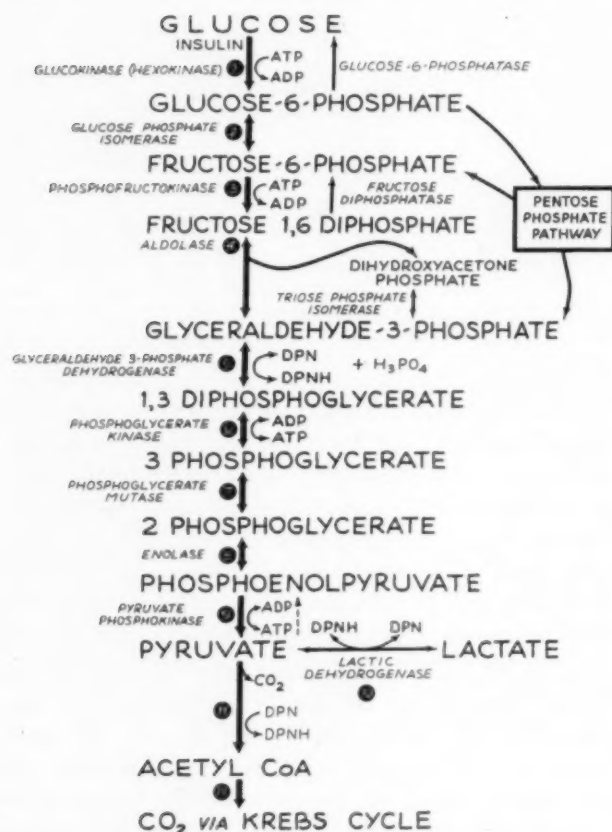


FIG. 1. Embden-Meyerhof pathway.

during which DPNH is reoxidized to DPN. On the other hand, in the absence of oxygen, the Krebs cycle cannot operate, and DPN may then be regenerated anaerobically by the conversion of pyruvate to lactate (Reaction 10, Fig. 1).

**Pentose Phosphate Pathway.** The major alternate mechanism by which glucose can be broken down is known as the pentose phosphate pathway, or hexosemonophosphate shunt. The relationship of this pathway to the Embden-Meyerhof route is shown in Figure 1, and the details of the reactions and enzymes involved are illustrated in Figure 2. In these reactions, glucose-6-phosphate instead of being converted to fructose-6-phosphate is oxidized by glucose-6-phosphate dehydrogenase to 6-phosphogluconolactone. Of major importance is the fact that triphosphopyridine nucleotide (TPN), rather than DPN, is the co-factor mediating this as well as the other oxidative reaction of the pentose phosphate route. 6-Phosphogluconolactone is next catalytically hydrated by the enzyme, lactonase, to yield 6-phosphogluconate. At this point, the first carbon of 6-phosphogluconate is removed as  $\text{CO}_2$  with the resultant formation of ribulose-5-phosphate. TPN again serves as the

oxidizing agent for this reaction. Two molecules of ribulose-5-phosphate are next converted (Reaction A and B, Fig. 2) to two closely related sugars, xylulose-5-phosphate and ribose-5-phosphate. Following this, the ten carbons represented by these two five-carbon molecules undergo a series of rearrangements, the first of which (Reaction C) is catalyzed by the enzyme, transketolase, and involves transfer of the first two carbons of xylulose phosphate to the ribose phosphate yielding a seven-carbon phosphorylated sugar known as sedoheptulose phosphate and a three-carbon sugar, glyceraldehyde phosphate. It is noteworthy that thiamine pyrophosphate is required as the coenzyme for this reaction. The first three carbons of sedoheptulose-7-phosphate are then transferred (Reaction D) to the glyceraldehyde phosphate to produce a six-carbon molecule, fructose phosphate and a four-carbon molecule, erythrose phosphate. The fructose phosphate now enters the Embden-Meyerhof pathway where it can either be converted to glucose or catabolized to pyruvate. As pictured in Reaction E, Figure 2, the nine carbons represented by the erythrose-4-phosphate plus another molecule of xylulose phosphate can undergo transketolation to yield fructose-6-phosphate and glyceraldehyde phosphate, both of which can enter the Embden-Meyerhof pathway to be further metabolized as shown in Figure 1.

The net result of the metabolism of glucose over the pentose phosphate pathway is indicated by the framed molecules in Figure 2. Of the eighteen carbons represented by the original three molecules of glucose-6-phosphate, twelve are utilized to produce two molecules of fructose-6-phosphate, three are converted to glyceraldehyde phosphate, and the remaining three carbons are oxidized to carbon dioxide.

The major differences in the pyridine co-factor requirements of the two pathways are emphasized in Figure 3. Both pathways of glucose breakdown may yield glyceraldehyde-3-phosphate; however, in doing so the Embden-Meyerhof route utilizes no pyridine co-factor while the pentose phosphate pathway requires TPN and yields TPNH. In addition, in the pentose phosphate pathway, thiamine pyrophosphate is necessary for both of the transketolase reactions. The fructose-6-phosphate and the glyceraldehyde phosphate which are produced by either the pentose phosphate or the Embden-Meyerhof mechanisms utilize DPN and produce

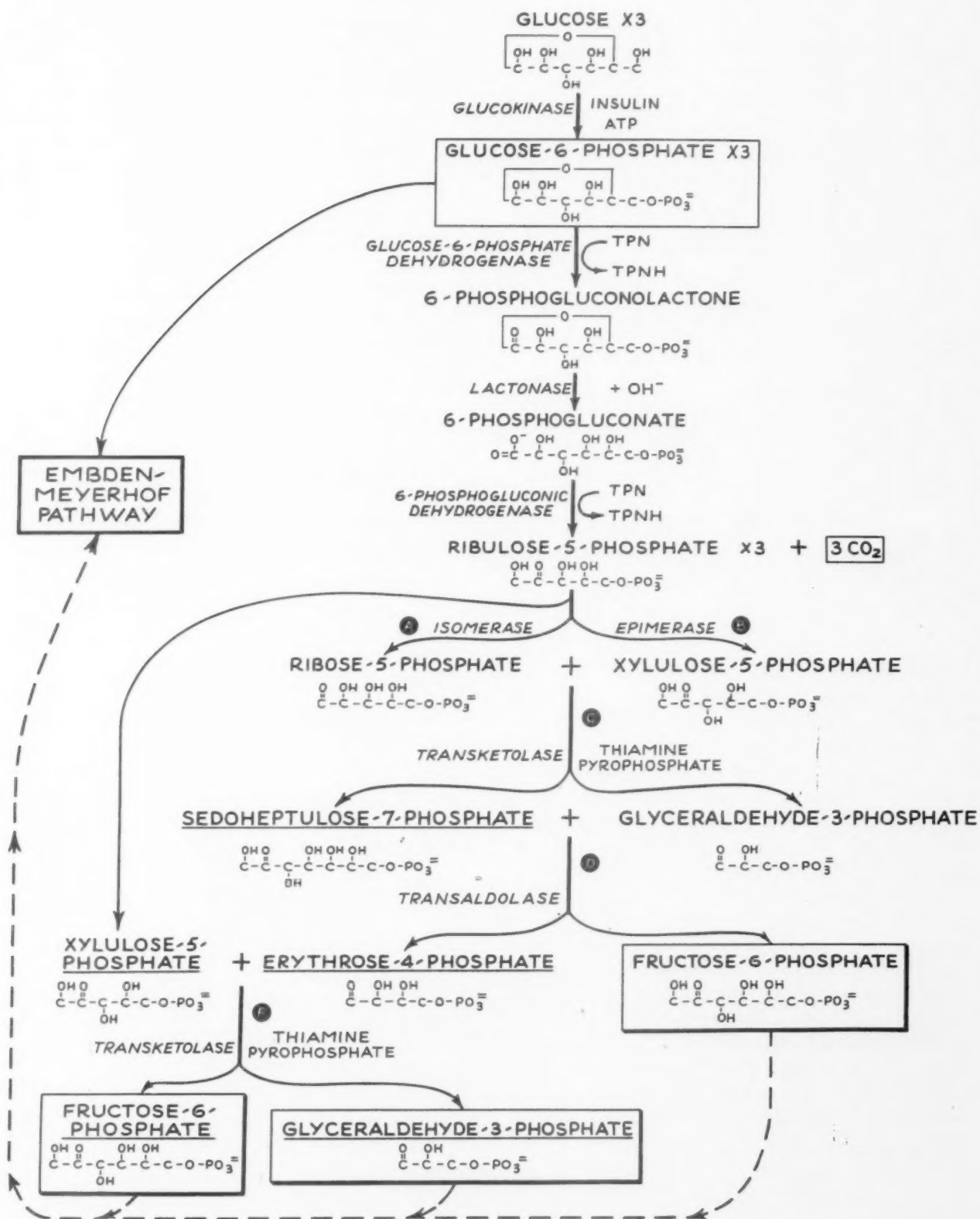


FIG. 2. Pentose phosphate pathway.

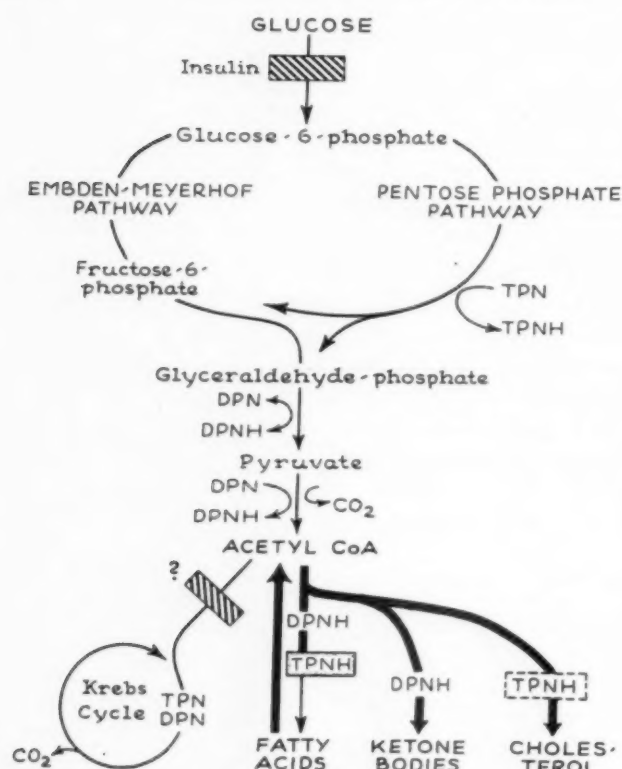


FIG. 3. Pathways of glycolysis and the effect of decreased glucose breakdown on lipid metabolism.

DPNH when further metabolized to pyruvate over the Embden-Meyerhof route.

**Quantitative Relationships of the Two Pathways.** Numerous attempts have been made to evaluate the relative quantitative importance of the two pathways of glucose breakdown. Most of these studies have been concerned with this relationship in the liver, and in this organ it has been found that from less than 10 to as much as 50 per cent of glucose breakdown takes place over the pentose phosphate pathway [9-12]. It should be noted, however, that in the intact perfused liver 56 per cent [13] and in the liver *in situ* 28 and 38 per cent [14] of glucose oxidation may occur over the pentose phosphate route. In certain other tissues, such as mammary gland [15] and adipose tissue [16], the pentose phosphate pathway may represent an even more important route of glucose metabolism. In muscle, on the other hand, all glucose breakdown probably takes place by way of the Embden-Meyerhof pathway [17].

The factors which control the proportion of glucose utilizing the Embden-Meyerhof and pentose phosphate pathways are of considerable importance since, as will be discussed, changes in the relative activities of these routes appear

to exert a marked influence on lipid metabolism. The level of glucose-6-phosphate dehydrogenase, the first enzyme involved in the pentose phosphate reactions, is no doubt one of the factors regulating this route of glucose breakdown. The activity of glucose-6-phosphate dehydrogenase has been found to vary greatly in different tissues, and a correlation can be shown between the level of this enzyme and the proportion of glucose utilizing the pentose phosphate route in normal liver [5,18], liver from pregnant rats [12,19], in muscle [17,18], mammary gland [15,19], and adipose tissue [16,20]. Furthermore, it has been clearly demonstrated that in diabetes, in which the amount of glucose utilizing the pentose phosphate pathway is depressed [21], the level of glucose-6-phosphate dehydrogenase activity is likewise greatly decreased [22]. Finally, in normal animals the activity of the enzyme has been found to be markedly and rapidly influenced by the amount of glucose in the diet, in that the livers of rats fed a high glucose diet contain approximately three to ten times the amount of glucose-6-phosphate dehydrogenase found in animals maintained on a low glucose intake [23,24].

Another factor of importance in determining the fate of catabolized glucose may be the intracellular level of the coenzymes, TPN and DPN. The addition of DPN to a homogenate of liver will enhance glucose oxidation over the Embden-Meyerhof pathway [25-27]. On the other hand, as might be predicted from Figure 3, addition of TPN to such a system will greatly stimulate pentose phosphate oxidation [26]; in fact, the pentose phosphate route can be made the dominant pathway of glucose breakdown if the level of TPN is sufficiently elevated [27]. This suggests that the normally very low level of TPN within the cell [28] may limit the amount of glucose utilizing the pentose phosphate route. Additional evidence that the concentration of TPN may be a critical factor in regulating glucose breakdown in the *intact* cell is provided by the finding that an artificial hydrogen acceptor such as methylene blue, which will oxidize TPNH to TPN, will also cause a significant increase in the amount of glucose utilizing the pentose phosphate route in liver [29] and in red cells [30].

Finally, a depressed level of the thiamine pyrophosphate required in the pentose phosphate route can, under certain circumstances, limit the operation of this pathway [37]. Such a defect has been well documented in Wernicke's syndrome



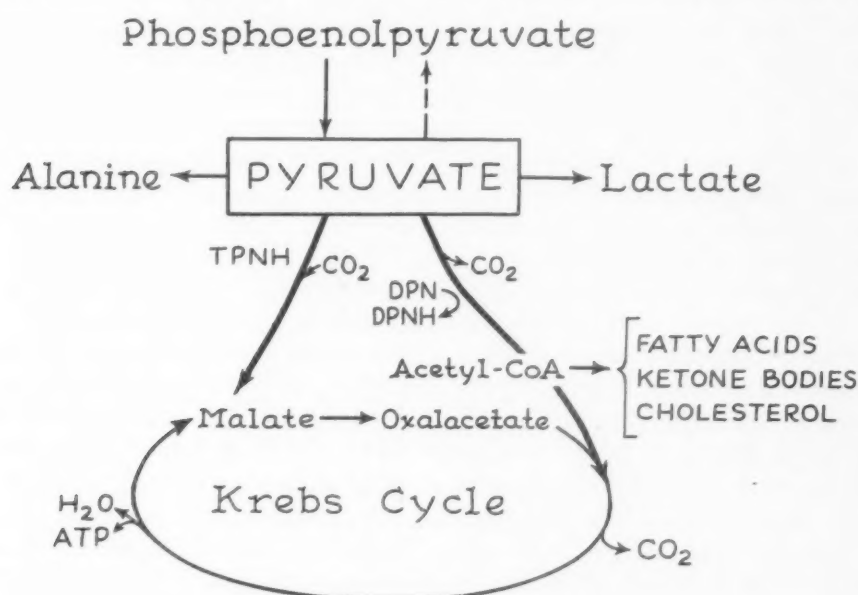


FIG. 4. Pathways of pyruvate metabolism.

[32]; however, there is as yet no evidence that thiamine plays a role in controlling the route taken by glucose breakdown when the diet is normal.

#### THE METABOLISM OF PYRUVATE

Following its formation from glucose, pyruvate, as shown in Figure 4, may undergo a number of metabolic fates, most of which have a bearing on lipid metabolism. The most important of the reactions of pyruvate are its decarboxylation to form acetyl CoA and its carboxylation to yield malate. The former requires the co-factors, thiamine pyrophosphate, lipoic acid, DPN and coenzyme A [33], while the fixation of  $\text{CO}_2$  to form malate requires TPNH as a co-factor [34]. Both of these processes play vital roles in the metabolism of glucose as well as of the lipids. Except for the carbon removed in the pentose phosphate pathway, the only mechanism by which glucose can be oxidized to carbon dioxide is by the decarboxylation of pyruvate and the oxidation of the resulting acetyl CoA in the Krebs cycle. Likewise, the major mechanism by which glucose is stored in the body involves the condensation of these acetyl CoA molecules to form fatty acids.

On the other hand, the conversion of pyruvate to malate serves the very important function of replenishing the dicarboxylic acids which are necessary for the functioning of the Krebs cycle. Finally, both hepatic gluconeogenesis and the net synthesis of amino acids are dependent upon

this route of pyruvate metabolism for their operation [35].

Little is known, as yet, concerning the factors which control these pathways of pyruvate utilization. It has generally been assumed that most of the pyruvate is decarboxylated to acetate either to be burned to carbon dioxide or to be stored as fatty acids; however, recent studies by Hill et al. [36] have suggested that as much pyruvate is carboxylated to form malate as is decarboxylated to yield acetate. The recent report of Freedman et al. suggests the important possibility that food intake may determine which of these two reactions may predominate [37]. In the animal fed a high glucose diet, pyruvate was found to be largely decarboxylated to acetyl CoA, while in the fasted animal pyruvate was diverted to malate synthesis. Such a mechanism would allow large amounts of glucose to be stored as fat when glucose is abundant; on the other hand, in the presence of limited dietary glucose, the preferential conversion of pyruvate to malate would provide the cell with a means of maintaining its Krebs cycle relatively intact despite a diminished supply of pyruvate.

Finally, thiamine may become a limiting requirement in the decarboxylation of pyruvate as evidenced by the depression of pyruvate oxidation seen in beriberi [38].

#### CONVERSION OF GLUCOSE TO FATTY ACIDS

It is now well established that the major mechanism by which the body is able to store

the calories of glucose is by the conversion of acetyl CoA to long-chain fatty acids. The fact that glucose could be converted to fatty acids in higher animals was strongly suggested by the balance studies and extensive respiration quotient data of the nineteenth and early twentieth centuries [39,40]; and with the advent of isotopic labeling, the conversion of glucose to fat could be demonstrated unequivocally [41]. The quantitative importance of this process in the storage of dietary glucose, however, was first established by the studies of Stetten and Boxer in 1944 [42]. These investigators used deuterium-labeled water to demonstrate that in rats fed a high glucose diet only a small amount of the stored glucose could be accounted for as glycogen, and they calculated that approximately 90 per cent of the glucose carbons which are retained in the body must be converted to fat. It is noteworthy that the studies of Drury [43] had earlier indicated that the conversion of carbohydrate to fat could be of vital importance to the organism. Drury found that normal animals fed every second day were readily able to maintain their body weights, a finding which suggested that during the days of feeding, calories were stored in amounts adequate to supply the animals' needs during the alternate days of starvation. Of the other hand, diabetic animals were unable to store carbohydrate under these circumstances and rapidly lost weight. Since diabetic rats which were fed *daily* remained in good health, it is apparent, as Drury suggested, that the diabetic has simply lost the ability to store carbohydrate, and it follows that, at least under circumstances of intermittent feeding, the storage of carbohydrate as fat is necessary for survival.

More recent studies using carbon-14 labeled glucose have confirmed these earlier conclusions and have directly demonstrated that the conversion of glucose carbons to fat in the intact animal is a rapid and quantitatively important process. Masoro et al. [41] have found that twenty-four and forty-eight hours after glucose ingestion, from 26 to as much as 100 per cent of the stored glucose of the mouse can be accounted for as fatty acids. Favarger and Gerlach [44] report that 12 minutes after injecting labeled glucose, as much as 3 per cent of the *total* glucose administered is found as fat.

*Site of Fatty Acid Synthesis in the Body.* A large number of studies have been performed in the past twenty years in an attempt to compare the

relative abilities of various tissues to synthesize fatty acids. It was soon found that slices of most tissues of the body are capable of incorporating either acetate-C<sup>14</sup> or glucose-C<sup>14</sup> into fatty acids; however, while lipogenesis could be readily demonstrated in intestine [41], kidney [45], diaphragm [45] and aorta [46], the amount of C<sup>14</sup> incorporated into fatty acids was consistently found to be greater in the liver than in other tissues. It was therefore generally concluded that the major site of fatty acid synthesis in the body was the liver [47], and it was assumed that the fat synthesized in the liver was then transported to the fat depots of the body for storage. It has become increasingly clear in recent years, however, that the liver must in fact play a relatively minor role in lipogenesis. This was first suggested by a comparison of turnover rates of fatty acids in the liver with that in the total animal, since it can be calculated from the figures given by Stetten and Boxer [42] that only about 4 per cent of the fatty acids synthesized in the body each day can be accounted for by hepatic lipogenesis. Further evidence was provided when Masoro et al. were able to demonstrate in the intact animal that less than 5 per cent of the fatty acids derived from an injected dose of glucose-C<sup>14</sup> could be found in the liver. Consistent with this finding is the fact that hepatectomized animals were capable of synthesizing fat at a rate comparable to that of an intact animal [41].

Overwhelming evidence has now accumulated to demonstrate that the major site of conversion of carbohydrate to fat is in the adipose tissue of the body. Shapiro and Wertheimer were the first to show that isolated adipose tissue was capable of synthesizing fatty acids [48]; and by the use of C<sup>14</sup>-labeled acetate and glucose, Feller [49] and Hausberger et al. [50] were able to demonstrate that slices of adipose tissue convert glucose to fatty acids at a rate faster than that of liver. This, coupled with the fact that the adipose tissue of man weighs approximately six times more than the liver [51], suggests that the major site of fat synthesis is indeed in the fat tissue itself. Favarger [44,52] has carried out studies in intact mice which strongly support this conclusion. Less than 4 per cent of the lipogenesis of the mouse was found to take place in the liver, and a comparison of the specific activities of the fatty acids obtained from various tissues indicates that almost all of the remaining fatty acid synthesis was carried out in the adipose tissue.

*The Intracellular Sites and Pathways of Lipogenesis.*

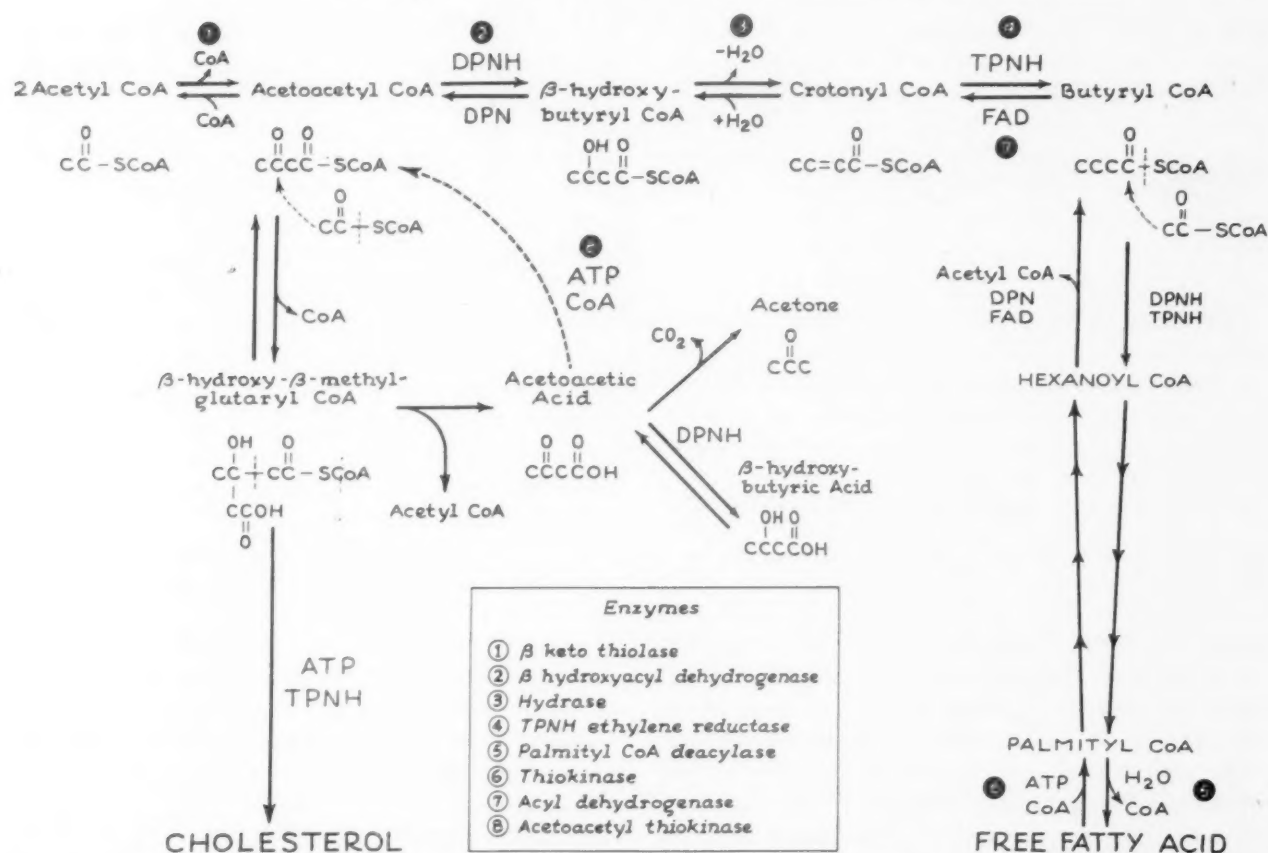


FIG. 5. "Mitochondrial" fatty acid synthesis and catabolism: their relationship to cholesterol and ketone body synthesis.

Studies performed within the past three years have demonstrated that long-chain fatty acids must be synthesized within the cell by two apparently independent processes. While the cytologic separation of these two mechanisms of lipogenesis is not yet definitely established, it appears likely that one series of reactions is located primarily in the mitochondria, and the other in the soluble supernatant fraction of the cell.

Fatty acids can probably be synthesized in the mitochondria of the cell by a process which is similar to but not identical with that used by the mitochondria for the oxidative breakdown of fatty acids [53]. This series of reactions, which will be termed "mitochondrial lipogenesis," is shown in Figure 5. The first step in this process involves the condensation of two molecules of acetyl CoA to form one of acetoacetyl CoA. In the synthesis of fatty acids, this compound is next reduced by a molecule of DPNH to form β-hydroxybutyryl CoA. As indicated by Reaction 3, β-hydroxybutyryl CoA can be dehydrated to form crotonyl CoA through the action of an enzyme known as hydrase, and finally the corresponding activated fatty acid is formed by the

reduction of crotonyl CoA (Reaction 4) to produce butyryl CoA. The latter reaction is of particular importance since it appears to represent one of the two steps by which the "mitochondrial" synthesis of fatty acids differs from the oxidative breakdown of fatty acids. In the oxidative process, flavine adenine dinucleotide (FAD) serves as the electron acceptor [54] whereas the source of hydrogen for the reduction on the synthetic pathway is provided by TPNH [55,56]. The microsomes of the cell may play a role in this reduction [57].

A six-carbon fatty acid may next be synthesized from butyryl CoA by the addition of a molecule of acetyl CoA to form the corresponding β-keto compound, which then undergoes reduction with DPNH, dehydration and finally reduction by TPNH to yield hexanoyl CoA. Repetition of this series of reactions occurs until long-chain fatty acid esters such as palmityl CoA are produced. Free fatty acids may then be formed by the hydrolytic removal of CoA, or, on the other hand, triglycerides or phospholipids may be synthesized by condensation with α-glycerophosphate [58,59].

In addition to the pathway of fatty acid



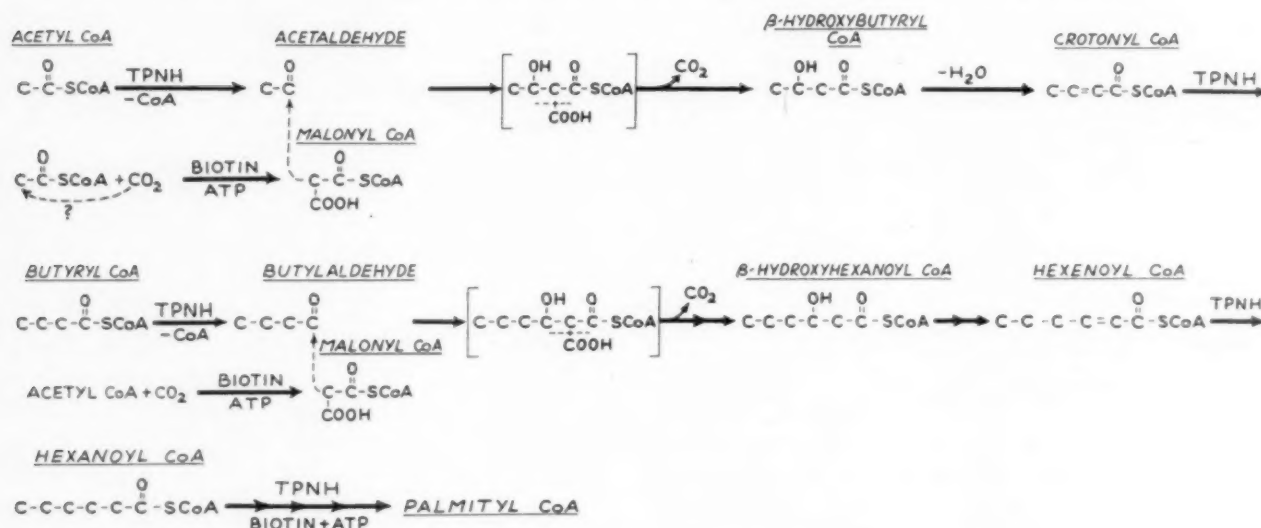


FIG. 6. Probable mechanism of fatty acid synthesis in "supernatant" fraction of cell cytoplasm. (After BRADY, R. O. [60].)

synthesis just described, the liver cell has recently been shown to possess a second mechanism of lipogenesis, which appears to be confined strictly to the supernatant fraction of the cell. The probable steps of this process, as suggested by Brady [60], are shown in Figure 6.

Langdon was the first to show that the cytoplasm of liver, when freed of particulate components, could still synthesize long-chain fatty acids if properly supplemented with co-factors [55]. It is possible, as Dituri et al. have suggested [67], that the lipogenesis which Langdon observed was due to the fact that the supernatant fraction as isolated by him contains a portion of the mitochondrial enzymes. Studies of Wakil et al. [62,63] have, however, clearly shown that the lipogenic reactions of the supernatant can be separated from the enzymes characteristic of mitochondrial lipogenesis. The major differences in co-factor requirements between the two mechanisms of fatty acid synthesis are that the mitochondrial system utilizes one molecule of DPNH and one of TPNH for each acetyl CoA added to the fatty acid chain [53] whereas, as Wakil et al. have demonstrated, the addition of each acetyl CoA in the supernatant system involves *two* molecules of TPNH [64,65] and no additional DPNH requirement has been observed [63]. In addition, adenosine triphosphate [64], biotin [66] and carbon dioxide [63] were found to be necessary for significant incorporation of  $\text{C}^{14}$ -labeled acetyl CoA into long-chain fatty acids by the supernatant system. The role of these co-factors was difficult to explain, especially since  $\text{C}^{14}\text{O}_2$  is not incorporated into

the synthesized fatty acids [63]. Brady has recently demonstrated that malonyl CoA is an intermediate in this process, and based on this finding he has suggested a very plausible mechanism for lipogenesis in the supernatant fraction of liver [60].

As shown in Figure 6, acetyl CoA again serves as the source of carbons for this route of fatty acid synthesis. Through the action of TPNH, one molecule of acetyl CoA is reduced to form acetaldehyde, while a second molecule of acetyl CoA is converted to malonyl CoA by the fixation of carbon dioxide on its methyl carbon. A condensation next takes place between the second carbon of the malonyl CoA and the carbonyl carbon of the acetaldehyde. The recently acquired carboxyl carbon of malonate is probably simultaneously split off, and  $\beta$ -hydroxybutyryl CoA is thereby formed. Butyryl CoA is then presumably synthesized from  $\beta$ -hydroxybutyryl CoA by dehydration and reduction with TPNH in a manner similar to that of the mitochondrial system; however, the enzymes involved may not be identical [63]. A six-carbon fatty acid would then be formed by the reduction of butyryl CoA to butyraldehyde, followed by the condensation of another molecule of malonyl CoA to yield  $\beta$ -hydroxyhexanoyl CoA plus carbon dioxide. The coenzyme A derivatives of long-chain fatty acids are thus produced in this scheme by successive condensations of aldehydes with malonyl CoA.

Brady's finding that malonyl CoA is an intermediate in lipogenesis is supported by the recent report of Wakil that when acetyl- $\text{C}^{14}$  CoA is

incubated with his enzyme system [67], a labeled derivative of malonate can be isolated. The role of ATP probably involves its known function in the activation of carbon dioxide [68]. Biotin, too, undoubtedly plays a role in the carbon dioxide fixation step of these reactions. This vitamin has long been implicated in carbon dioxide fixation reactions [69] and recently has been shown to be specifically required in the transfer of the carboxyl group from "active carbon dioxide" [70]. The exact role of these co-factors and the details of the reactions involved in lipogenesis by the "supernatant" scheme have yet to be confirmed; nonetheless, at present it is safe to conclude that both mechanisms of fatty acid synthesis exist within the liver cell. Which of these processes is quantitatively the more important in liver, and especially in adipose tissue, has not, however, been established.

*The Influence of Glucose Breakdown on Fatty Acid Synthesis.* There is abundant evidence to support the conclusion that the pathways of lipogenesis just described are markedly influenced by the rate of glucose breakdown. The feeding of a diet high in glucose causes a striking stimulation in the rate of fatty acid synthesis both from acetate and from glucose [23,71]. On the other hand, fasting produces a prompt depression of lipogenesis [23,71-74], and this effect can be reversed specifically with glucose [75,76]. It is also noteworthy that lipogenesis in adipose tissue [77], liver [75,78] and mammary gland [79] can be significantly stimulated simply by the addition of either glucose alone or glucose plus insulin to slices of the respective tissues.

Furthermore, the defect in glucose catabolism of diabetes is characteristically accompanied by an almost complete inability to convert either glucose or acetate to fatty acids in the intact animal [80,81], in liver [82-84], and in adipose tissue [50]. Although the diabetic defect in fatty acid synthesis is readily repaired by administering insulin [87], this lipogenic lesion is definitely *not* due to an insulin requirement in the reactions of fatty acid synthesis but is in fact *secondary* to the depressed glycolysis, as has been shown in the intact animal [85], in liver homogenates [86], and most clearly in adipose tissue slices [77].

It is apparent from the results of these many studies that glycolysis must in some manner be specifically required for lipogenesis to proceed at an optimal rate.

The question next arises as to the mechanism

by which glucose breakdown exerts so marked an effect on the synthesis of fatty acids. Since, as noted earlier, lipogenesis represents the major mechanism by which glucose is stored in the body, one might reasonably expect that in the absence of glycolysis the carbon substrates necessary for lipogenesis might be reduced to the point where fatty acids could no longer be synthesized. This, however, is clearly not the case. In the first place, as will be discussed later, there is reason to believe that the substrate for fatty acid synthesis, acetyl CoA, is present in normal or perhaps even excessive amounts in both the starved and the diabetic animal. In the second place, the studies with C<sup>14</sup>-labeled acetate [83,84] demonstrate that glycolysis must have its primary influence on lipogenesis at some point in this synthetic pathway after the formation of acetyl CoA. It is therefore apparent that the primary function of glucose in enhancing lipogenesis cannot be simply to supply the substrate for this reaction but, rather, it would appear that some compound formed during the breakdown of glucose to acetyl CoA serves to stimulate the conversion of acetyl CoA to long-chain fatty acids.

Since the process of glucose breakdown can take place over either the Embden-Meyerhof pathway or the pentose phosphate route, it is reasonable to ask whether one or the other of these pathways is primarily responsible for the stimulatory effect of glycolysis upon lipogenesis. There are at present three distinct lines of evidence which would support the view that, although the Embden-Meyerhof pathway is quantitatively the more important route of glucose breakdown in most animal tissues, it is the glucose utilizing the pentose phosphate pathway which is primarily responsible for enhancing lipogenesis.

First, experimental conditions which stimulate lipogenesis appear also to stimulate pentose phosphate oxidation. Tepperman and Tepperman have very nicely demonstrated that following the feeding of glucose loads to rats, the prompt increase in lipogenesis is accompanied by a marked rise in the activity of the pentose phosphate enzymes [88]. In addition Felts et al. have directly shown with differently labeled glucose that the administration of insulin to diabetic animals causes both a preferential increase in glucose oxidation over the pentose phosphate route and a concomitant increase in hepatic lipogenesis [89]. Similarly Winegrad

and Renold have demonstrated that the addition of insulin greatly stimulates both glucose oxidation and lipogenesis of adipose tissue slices from normal rats [77], and it is noteworthy that the marked increase in the amounts of  $\text{CO}_2$  originating from the first carbon of the glucose would suggest that a major portion of the stimulation of glycolysis occurs over the pentose phosphate pathway [90].

Second, a good correlation has been found between the ability of various tissues of the body to synthesize fatty acids and the activity of the pentose phosphate pathway in that tissue. As noted earlier, the major site of lipogenesis in the body is the adipose tissue and, as has been shown by Milstein et al. [16], pentose phosphate activity is probably greater in this tissue than in liver. Under conditions of lactation, the mammary gland represents an extremely active site of lipogenesis [79] and Abraham et al. have demonstrated that this tissue, too, contains a powerful pentose phosphate pathway, which may in fact be of greater quantitative significance than the Embden-Meyerhof route [15]. Conversely, tissues such as brain, which synthesize fatty acids poorly, contain little or no pentose phosphate activity [91].

Finally, since the addition of DPN to a liver homogenate stimulates the Embden-Meyerhof pathway whereas TPN accelerates the pentose phosphate route [25-27], the relative influence of these two pathways on fatty acid synthesis can be determined by measuring the rate of lipogenesis during selective stimulation of one or the other glycolytic pathway. The results of such studies [92,93] have indicated that, while oxidation of glucose via the Embden-Meyerhof pathway causes only a modest increase in the amount of fat synthesized, stimulation of glucose via the pentose phosphate route results in a very marked enhancement of lipogenesis, which on the average amounts to approximately 100 times that observed in homogenates not carrying out active glycolysis. The Embden-Meyerhof pathway may, under certain circumstances, play a secondary role in the stimulation of lipogenesis since in the presence of active pentose phosphate glycolysis the addition of DPN will cause a further enhancement of fatty acid synthesis.

These three types of evidence would therefore indicate that glucose breakdown via the pentose phosphate route is primarily responsible for the stimulatory effect of glycolysis on lipogenesis. Such a relationship would provide the cell with

a useful regulatory mechanism, in that dietary glucose in excess of that required for immediate energy might be preferentially directed into the pentose phosphate route, and the consequent stimulation of lipogenesis would then insure the storage of this glucose as fat.

The means by which pentose phosphate glycolysis is able to effect an enhancement of lipogenesis is suggested by the differences in co-factors produced by the two pathways of glycolysis. As emphasized in Figure 3, the operation of the pentose phosphate pathway results in the generation of TPNH, and if the fructose-6-phosphate and glyceraldehyde phosphate produced are subsequently converted to pyruvate, DPNH will also be produced. On the other hand, the operation of the Embden-Meyerhof pathway can result only in the generation of DPNH. In view of the fact that TPNH is required for both the mitochondrial and supernatant mechanisms of lipogenesis, it seemed likely that TPNH might be the factor mediating the stimulatory effect of the pentose phosphate route on lipogenesis. Direct evidence that TPNH does in fact serve this function is provided by the finding that in liver homogenates a non-glycolytic TPNH generating system, isocitrate and TPN, also stimulates lipogenesis to a very marked degree [56,93]. It would follow that although the DPNH and ATP produced during the operation of the latter portion of the Embden-Meyerhof pathway are both definitely required for fatty acid synthesis, these co-factors must normally be supplied in sufficient quantities so that they are not limiting in the process of lipogenesis. Additional evidence that TPNH is in fact the crucial co-factor in maintaining lipogenesis is provided by the finding of Cahill et al. that methylene blue, a compound which will remove TPNH by catalyzing its oxidation to TPN, greatly depresses lipogenesis in the intact liver cell [29].

Finally, Catravas and Anker have recently demonstrated that normal mitochondria contain a heat-stable factor which will stimulate lipogenesis in the liver of fasted rats [94]. The nature of this substance, termed "lipogenin," is unknown; however, it appears to possess activity in the intact animal [95] and may play a role in the physiological regulation of lipogenesis.

In summary, then, it is definitely established that fatty acid synthesis is greatly influenced by the rate at which glucose is broken down in the body, and present evidence would indicate that the glucose oxidized over the pentose phosphate



pathway is primarily responsible for this ability of glucose catabolism to stimulate lipogenesis. The major factor mediating this effect of pentose phosphate oxidation is probably the reduced triphosphopyridine nucleotide generated during glucose oxidation over this pathway. The intrinsic ability of various tissues of the body to generate TPNH may therefore determine the rate of lipogenesis in these tissues; likewise, TPNH generation would seem to be the primary mechanism by which the body controls the rate of lipogenesis during normal variations in carbohydrate ingestion. Finally, it would follow that the likely site of control of lipogenesis, at least in the mitochondria, is located at the irreversible ethylene reductase reaction (Reaction 4, Fig. 5) involving the conversion of crotonyl CoA to butyryl CoA.

#### FATTY ACID OXIDATION

The intracellular site of fatty acid oxidation has been clearly localized to the mitochondria [96] and, as noted earlier (Fig. 5), in all respects but two, this process appears to consist of the reversal of the biochemical reactions involved in the mitochondrial synthesis of fatty acids. Whereas the rate-limiting conversion of crotonyl CoA to butyryl CoA utilizes TPNH as the hydrogen donor, the reverse reaction (Fig. 5, Reaction 7), involving the oxidation of the fatty acid by acyl dehydrogenase, utilizes flavin adenine dinucleotide rather than TPN as a hydrogen acceptor [54]. Secondly, the activation of free fatty acids to form palmityl CoA (Reaction 6, Fig. 5) requires adenosine triphosphate [97], and is catalyzed by an enzyme, thiokinase, while the formation of free fatty acids from palmityl CoA on the synthetic pathway is a hydrolytic process yielding no high-energy bonds [53]. It is also noteworthy that despite the fact that all of the oxidative steps of fatty acid breakdown are localized in the mitochondria, the initial activation of the free fatty acid takes place in the supernatant and microsomal fractions of the cell [97]. This would suggest that palmityl CoA must be formed from palmitic acid extramitochondrially and is then transferred into the mitochondria to be broken down to acetyl CoA.

*The Influence of Glucose on Fatty Acid Oxidation.* There is no longer any doubt that glucose breakdown exerts a definite influence on the oxidation of fatty acids as well as on their synthesis. Older studies involving measurements of the respiratory quotient provided some indication that

when animals were fasted, an increase in fat utilization from the fat depots occurred [39,98]. Similarly, in the diabetic, the R.Q. was usually found to be depressed, suggesting that with decreased glucose utilization a greater dependence on fat breakdown occurred [39]. Unequivocal evidence that fatty acid oxidation is intimately interlinked with the supply of glucose has been provided recently by the studies of Lassow et al. [99,100]. By following the conversion of  $C^{14}$ -labeled tripalmitin to  $C^{14}O_2$  these authors have been able to demonstrate that glucose exerts a significant sparing effect on the rate of tripalmitin oxidation. Furthermore, this effect can be demonstrated *in vitro*, in that liver slices prepared from glucose-fed rats oxidized tripalmitin at about one-half the rate of slices prepared from fasted rats [99]. A similar effect has been reported by the same authors in diabetic animals, and in this case, too, the abnormally rapid oxidation of fat could be reversed by increasing the rate of glucose oxidation with insulin [100].

At the present time there appear to be at least two mechanisms by which glucose is able to decrease the rate of fatty acid oxidation. It is probable that the immediately utilizable form of fat is the mixture of free fatty acids formed in the adipose tissue and transported as such to various sites of oxidation [101,102]. The level of free fatty acids within the blood may therefore be used as an index of mobilization of depot fat for oxidative purposes. Two groups of investigators have been able to demonstrate that starvation increases and the administration of glucose depresses the concentration of free fatty acids in the blood [101-103]. Furthermore, this action of glucose appears to be localized to the mechanism in the adipose tissue for converting triglyceride into free fatty acids, in that increased glucose utilization causes a decrease in the rate of release of non-esterified fatty acids from isolated slices of adipose tissue [104-107], while starvation or conditions causing decreased glucose uptake [107] result in accelerated formation of free fatty acids in the *in vitro* system [104-106]. Since high concentrations of free fatty acids can be found *intracellularly* in the latter state [106], glucose deprivation probably serves to activate a lipase which then splits fatty acids from the stored triglycerides. It should be mentioned that with severely depressed glycolysis, such as may be seen in diabetic ketosis and starvation, the increased mobilization of lipids may also in-

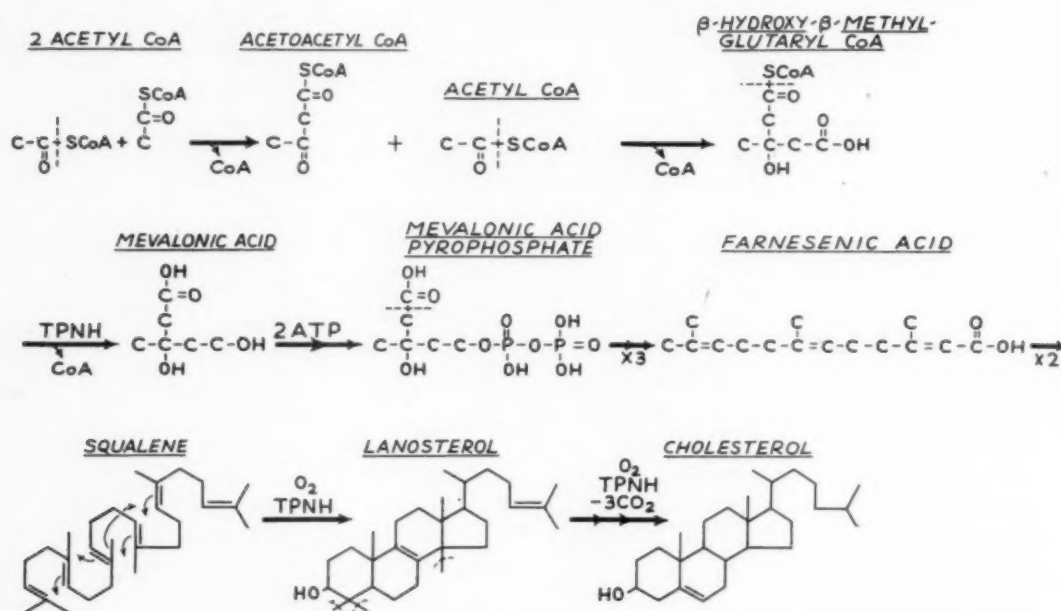


FIG. 7. Pathway of cholesterol synthesis.

clude triglycerides, and gross lipemia may result [108].

In addition to its action on the hydrolysis of triglycerides to free fatty acids, glucose must also exert a *direct* effect on the intracellular oxidation of free fatty acids to  $\text{CO}_2$  since the studies of Lassow et al. have demonstrated that *free* palmitic acid-1- $\text{C}^{14}$  is oxidized at increased rates in starved rats and at decreased rates when glucose is administered [99]. The mechanism by which glucose can so effectively depress fatty acid oxidation is at present completely unknown.

#### CHOLESTEROL SYNTHESIS

It was shown some years ago by Bloch and Rittenberg that the two-carbon unit, acetate, provides the major substrate for the synthesis of cholesterol [109]. Furthermore, it has been established that, with the exception of adult brain, this process can take place in every tissue of the body [110], including the arterial walls of at least the rabbit, the chicken [111] and the rat [112].

The biochemical steps involved in the synthesis of cholesterol from acetate have been the subject of intense investigation over the past five years, and as a result the major steps in this process are now fairly well understood. These reactions are summarized in Figure 7. As in the "mitochondrial" synthesis of fatty acids (Fig. 5), cholesterol synthesis is initiated by the condensation of two molecules of acetyl CoA to form one

of acetoacetyl CoA. At this point the two synthetic pathways diverge in that in the synthesis of cholesterol, acetoacetyl CoA is next attacked by a third molecule of acetyl CoA to yield the six-carbon compound,  $\beta$ -hydroxy- $\beta$ -methylglutaryl CoA [113]. The further reactions of this acid represent another important branching point in intermediary metabolism since, as shown in Figure 5,  $\beta$ -hydroxy- $\beta$ -methylglutaryl CoA can lead either to the synthesis of cholesterol or to the production of the ketone bodies, acetoacetic acid,  $\beta$ -hydroxybutyric acid and acetone [114]. In the synthesis of cholesterol, the carboxyl carbon of  $\beta$ -hydroxy- $\beta$ -methylglutaryl CoA is reduced by TPNH, and the CoA is removed to yield the corresponding alcohol, mevalonic acid [115,116]. Mevalonic acid can then react in sequence with two molecules of ATP [117] to yield mevalonic acid pyrophosphate [118], and this compound eventually results in the formation of a fifteen-carbon compound which may be farnesenic acid [119] or farnesene [120]. Two of these fifteen-carbon units then join to form the thirty-carbon structure of squalene, and squalene, as shown in the last line of Figure 7, can undergo ring closure to yield the cholesterol precursor, lanosterol [127]. Finally, the latter compound, through a series of some fourteen reactions requiring TPNH and oxygen, loses three of its carbons and one double bond to form cholesterol [122].

#### Effect of Glucose Oxidation on Cholesterol Synthesis.

The rate of glucose breakdown clearly has a marked effect on cholesterol synthesis. Tomkins and Chaikoff demonstrated in 1952 [123] that livers from fasted rats carry out cholesterol synthesis at greatly reduced rates. Since the administration of glucose alone was capable of restoring cholesterolgenesis to normal, it may be concluded that glycolysis must exert some specific effect in stimulating this process. This conclusion is supported by the fact that in severely diabetic animals, cholesterol synthesis may be depressed [83]; however, this relationship is complicated by the fact that cholesterolgenesis may also be markedly increased in diabetes [124].

The mechanism by which glucose breakdown influences cholesterol synthesis in this variable manner is not definitely known. Studies utilizing the selective stimulation of glucose breakdown via the Embden-Meyerhof or pentose phosphate pathways have indicated that the pentose phosphate route has by far the more important role in the glucose stimulation of cholesterolgenesis [93]. Enhancement of this pathway was found to produce an approximately 300-fold increase in the rate of cholesterol synthesis, while glycolysis via the Embden-Meyerhof pathway caused only an insignificant increase in this process. Of particular interest was the observation that when the Embden-Meyerhof route was stimulated simultaneously with the pentose phosphate pathway, a relative *depression* in cholesterol synthesis was produced.

A possible explanation for these observations becomes apparent on examining the relationship of cholesterol synthesis to lipogenesis shown in Figures 3 and 5. Since a TPNH generating system not utilizing glucose oxidation was found to stimulate cholesterol synthesis, it seems likely that the ability of pentose phosphate glycolysis to accelerate this process is due to the generation of TPNH. As indicated in Figure 7, TPNH is the only hydrogen source involved in the synthesis of cholesterol, whereas both DPNH and TPNH are utilized at least by the "mitochondrial system" of lipogenesis. (Fig. 5.) It has been suggested [93,125], therefore, that when rapid glycolysis occurs over the Embden-Meyerhof pathway concurrently with active pentose phosphate oxidation, the large supplies of DPNH produced may depress the synthesis of cholesterol by stimulating lipogenesis to the point that the precursors of cholesterol synthesis are depleted. Such an explanation of the depres-

sion of cholesterolgenesis produced by Embden-Meyerhof glycolysis is consistent with the interesting finding of Tepperman that, during glucose loading, cholesterol synthesis is depressed when fatty acid synthesis is most active [88].

It may be concluded, therefore, that glucose breakdown is required for optimal cholesterol synthesis; however, glycolysis via the pentose phosphate route is probably responsible for this effect, whereas glycolysis over the Embden-Meyerhof route may actually cause a depression of cholesterol synthesis.

#### KETOSIS

In such conditions as fasting or diabetes, or during high fat, low carbohydrate diets, glucose breakdown is decreased, and an accumulation of ketone bodies consistently occurs. It is now apparent that the mechanism by which a depression in glycolysis leads to ketosis is very complex. A decreased rate of ketone body utilization is probably not responsible for the development of ketosis [126]; on the other hand, there is good evidence that ketosis is the result of increased ketone body synthesis [127,128]. Isotopic studies have clearly established that the ketone bodies, acetoacetic acid,  $\beta$ -hydroxybutyric acid and acetone, must be derived from acetyl CoA [129], and it is therefore reasonable to assume that excessive ketone body synthesis is the result of an accumulation of acetyl CoA. An increase in acetyl CoA might in turn be due to either accelerated production or to decreased removal of this intermediate, since as noted earlier glycolysis can influence both the origin and the fate of acetyl CoA.

Impaired glycolysis definitely results in an increase in the rate at which fatty acids are oxidized [99], and under these circumstances the energy requirements of the cell are met by the acetyl CoA supplied from the fatty acids of either the diet or the depot fat. Since, in contrast to glucose, all of the carbons of fat must be converted to acetyl CoA before their oxidation to  $\text{CO}_2$ , it is possible that the total influx of acetyl CoA may actually be in excess of normal when fat is used as the major source of energy; however, this latter point is by no means proved.

The factors influencing the *disposal* of acetyl CoA probably play a larger role in the genesis of ketosis than do those involved with acetyl CoA production. As has been mentioned earlier, the two major pathways by which acetyl CoA is removed from the body are (1) oxidation to



carbon dioxide and water in the Krebs cycle, a process which represents the major energy source of the body; and (2) the storage of excess acetyl CoA by conversion to long-chain fatty acids. (Fig. 3.)

There is a good theoretical basis for suggesting that the rate of glucose breakdown may limit the ability of the Krebs cycle to dispose of the acetyl CoA molecules derived from fat. Under normal circumstances, there is a continuous attrition of the four-carbon molecules of the Krebs cycle, and, as was discussed in detail earlier, the major mechanism by which these compounds can be replaced is by carbon dioxide fixation to pyruvate, or perhaps to phosphoenolpyruvate [130], both of these compounds being products of glucose breakdown. When glycolysis is diminished, the levels of phosphoenolpyruvate and pyruvate may decrease, and the rate at which the four-carbon, Krebs cycle intermediates could be replaced would be depressed [137]. A reduction in the TPNH needed for CO<sub>2</sub> fixation with pyruvate might further contribute to this biochemical lesion. As a result of such a defect, acetyl CoA oxidation in the Krebs cycle would be decreased, and an accumulation of acetyl CoA within the cell should then occur. Eventually, due to a mass action effect, the increased concentration of acetyl CoA could allow the total amount of carbon dioxide produced by the Krebs cycle to attain normal limits; however, it should be emphasized that the total concentration of acetyl CoA under these circumstances must remain elevated. As pointed out earlier, the tendency of impaired glucose breakdown to reduce the level of the Krebs cycle intermediates might be in part ameliorated by the fact that the small quantities of pyruvate available under these circumstances would be preferentially utilized to form the Krebs cycle intermediates rather than being converted to acetyl CoA [37].

Numerous studies have been carried out in an attempt to determine whether this relationship of glycolysis to Krebs cycle oxidation is correct. Unfortunately, these have led in most cases to very conflicting results. Attempts to treat ketosis by the administration of Krebs cycle intermediates have been successful in some studies [128,131-133], but not in others [133-135]. The oxidation of acetate-C<sup>14</sup> to C<sup>14</sup>O<sub>2</sub> appears to be normal in the diabetic rat, suggesting that the Krebs cycle may not be defective in this animal under conditions of depressed glycolysis [136];

however, in the diabetic dog, a defect in the breakdown of acetate to CO<sub>2</sub> has been demonstrated [137]. Finally, attempts to measure the level of Krebs cycle intermediates directly have resulted in a report of markedly depressed levels of these compounds by Frohman [138], while, on the other hand, Shaw has recently found that the level of oxalacetate in the liver of alloxan-diabetic rats is normal [139].

It should be concluded, therefore, that although a defect in the oxidation of acetyl CoA in the Krebs cycle provides the best theoretical explanation for the appearance of ketosis during glucose deprivation, an influence of glucose on this aspect of acetyl CoA metabolism has by no means been proved.

The second mechanism by which excess acetyl CoA might be removed from the cell is by conversion to fatty acids, and this process, as documented earlier, is greatly impaired in any condition accompanied by reduced glucose oxidation. Since the availability of TPNH appears to be the glycolytic factor which usually limits lipogenesis, it would follow that in the absence of adequate glucose breakdown, mitochondrial lipogenesis would be blocked at the site of action of this coenzyme, i.e., at the conversion of crotonyl CoA to butyryl CoA [55] (Fig. 5), and the intermediates involved prior to this block would be expected to accumulate. Lynen et al. [114] have recently demonstrated that, contrary to previous concepts, the major pathway of acetoacetic acid synthesis is by removal of a molecule of acetyl CoA from  $\beta$ -hydroxy- $\beta$ -methylglutaryl CoA [140].  $\beta$ -Hydroxybutyric acid can then be formed by reduction of acetoacetic acid through the action of DPNH, while acetone is produced by spontaneous decarboxylation of acetoacetic acid. An accumulation of the lipogenic intermediates prior to butyryl CoA would be expected to cause an elevation in the level of acetoacetyl CoA and hence of the immediate precursor of the ketone bodies,  $\beta$ -hydroxy- $\beta$ -methylglutaryl CoA.

It seems reasonable to conclude, therefore, that ketosis is, in a sense, the passive consequence of an accumulation of acetyl CoA and the inability of this acetyl CoA to be metabolized beyond the level of crotonyl CoA on the pathway of lipogenesis. The concept that an elevated level of  $\beta$ -hydroxy- $\beta$ -methylglutaryl CoA is the immediate cause of ketosis is supported by the fact that cholesterol, which must also be derived from

$\beta$ -hydroxy- $\beta$ -methylglutaryl CoA, tends to be synthesized in increased amounts in a ketotic state such as diabetes.

## CONCLUSIONS

In summary, it is becoming increasingly apparent that fatty acid synthesis and oxidation, cholesterol synthesis, and ketone body accumulation all are in part controlled by the rate at which glucose is broken down within the cell. It has been the purpose of this review to outline the present concepts of the biochemical pathways of glucose breakdown and of lipid metabolism, and to indicate by what mechanisms glycolysis is able to exert so marked an influence on these many processes. In particular, it has been emphasized that glucose, in addition to serving the vital function of supplying substrate for the operation of Krebs cycle, acts as a generating system for the reduced pyridine nucleotides, and that it is through these coenzymes, and in particular TPNH, that glycolysis may be able to exert its regulatory influence on lipid metabolism.

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# Some Aspects of the Glucagon Problem\*

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**D**URING the last ten years it has frequently been suggested that glucagon is a hormone, and many investigations have been directed toward proving or disproving this hypothesis. Evidence in favor of this view has rapidly accumulated, but conclusive proof is still lacking. This uncertainty has not discouraged numerous workers from thoroughly investigating the chemistry of glucagon, its physiological action and the mechanism of its action.

Recent reviews [20,28,37,56] have presented a thorough discussion of the problems related to glucagon and provide a fairly up-to-date literature. The closely related subjects of insulin antagonists [36], epinephrine [41] and the mechanism of action of growth hormone [77] have also been extensively reviewed recently. The subject matter of the present paper has therefore been restricted to a detailed consideration of certain points which seem at the moment to be of a great theoretical or practical interest.

*Preparation and Assay.* New possibilities for research were opened in 1953 when a group of biochemists from the Lilly Research Laboratories succeeded in obtaining crystalline glucagon. This preparation, which has been used subsequently by many workers, is undoubtedly very pure. However, Randle [98] showed recently that some samples of crystalline glucagon are contaminated by about 1 per cent insulin, a larger amount than was previously believed to be present. According to Randle's data, the insulin content may vary from batch to batch and may be higher for cruder preparations. It should be mentioned in this connection that no insulin-inactivating step is included in the purification procedure for glucagon [114]. The biological assay for insulin may still be reliable in mixtures containing up to 30 per cent of glucagon [105], although this may depend on the method used [131]. It is unlikely, however, that any *in vivo* insulin assay would be valid in the presence of very large amounts of glucagon.

As Randle [98] could not detect any effect of glucagon *per se* on the rat diaphragm *in vitro*, the rat diaphragm method for the assay of insulin could be used in the future to obtain a more reliable estimate of the amount of insulin in samples of glucagon. A recent paper by Elrick [47] suggests that his previous experiments on the growth-promoting properties of glucagon [42] were vitiated by the impurities present in a crude glucagon preparation, although insulin was probably not responsible in this case for the observed effect [60]. It has also been observed that impurities may influence glucagon action, probably by delaying its resorption after intraperitoneal injection [85].

In consideration of these observations, earlier results obtained with crude preparations of glucagon should be interpreted with caution, and even recent ones may not all be beyond criticism. It seems that adequate control experiments should be run more frequently, particularly when large doses of glucagon are employed. Some well known properties may be applied to distinguish glucagon from insulin and possibly from other active proteins. Glucagon is very sensitive to proteolytic enzymes, but remains active after incubation in alkaline solution or with cysteine. Insulin, on the other hand, is rather resistant to proteolytic enzymes but is easily inactivated by alkali or cysteine.

The structure of glucagon was established in 1956 by the Lilly group [15]. The peptide of 29 amino acids has no obvious peculiarities. Its amino acid sequence is very different from that of insulin. At present, a purely chemical assay does not seem feasible, and the determination of glucagon therefore is based on its biological properties. The hyperglycemic response of the cat has been frequently employed, but more accurate and sensitive procedures have been described. Methods based on the increased output of glucose by liver slices [132] or the stimulation of phosphorylase activation in liver

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homogenates [10] are more accurate and well adapted to rigorous statistical treatment. However, the determination of glucagon in biological materials is still difficult, except in tissues such as pancreas where it is abundant. The assay in blood is impossible without purification. In this respect, an important advance was made recently by Makman, Makman and Sutherland who have devised a purification procedure which makes possible a reliable determination of glucagon in the plasma of dog or man [86]. This progress and further improvements in method should contribute much to the solution of many physiological and clinical problems concerning glucagon.

*Origin and Biological Inactivation.* Glucagon has been found in the pancreas of all vertebrates thus far investigated. The amount present is closely related to the number and integrity of the  $\alpha$ -cells. As no other cell type gives such good correlation, most authors favor the hypothesis that the  $\alpha$  cells are the only cellular constituent of the pancreas which contain glucagon. This controversial subject has been thoroughly discussed recently [28,37].

Other possible sites of origin have not received much attention; however, there is no conclusive evidence that they might not be as important physiologically as the  $\alpha$ -cells. The gastric mucosa of the dog, for example, contains considerable amounts of a hyperglycemic factor; smaller quantities are found in the duodenum [119]. The stomach of some species, such as cattle, is devoid of the factor. No detailed survey of the distribution of glucagon in the digestive tract of different species has been made, and no satisfactory hypothesis has been proposed in regard to the type of gastric or intestinal cells forming glucagon. This difficulty has prompted some authors to suggest that the gastric factor is an epinephrine-like compound, different from pancreatic glucagon. However, the factor can be purified from gastric mucosa by the same method that is used for the isolation of glucagon from pancreas [118], and it is about as sensitive as glucagon to proteolytic enzymes or to the insulinase system of liver [113]; further, its action on liver slices, like that of glucagon, is not blocked by ergotamine [133]. The gastric factor therefore would appear to be identical with or very similar to pancreatic glucagon. No substances resembling glucagon have been found except in the organs mentioned. Reports [99,100] that glucagon could be extracted from spleen, lymph nodes, skin or

tongue were not confirmed in this laboratory [133].

The short duration of the glucagon effect can probably be ascribed to its rapid destruction or fixation in tissues such as the liver or the kidney [6,32]. The nature of the proteolytic enzyme(s) inactivating glucagon is not known, but the system is not specific for this substance. Many peptides or proteins, such as insulin, ACTH or growth hormone, prevent the destruction of glucagon by liver tissue *in vitro*. Conversely, glucagon can inhibit insulin inactivation by rat diaphragm [90].

*Hyperglycemic-Glycogenolytic Properties.* The ability to induce a rapid and pronounced hyperglycemia is the most striking property of glucagon. There is little doubt that the extra glucose put into circulation originates primarily from the liver. The observation that glucagon acts also on rat skin [101] could not be reproduced in this laboratory [133]. Numerous studies indicate that the release of glucose from the liver is associated with a major breakdown of glycogen. A continuous glucagon infusion can almost completely deplete the glycogen stores of the liver [19]. However, twelve to twenty-four hours after a single dose of glucagon the liver glycogen is frequently increased [103,104]. Careful investigations by Foa and his group have established that the early and striking decline in hepatic glycogen is followed by an increase above the initial level [30]. A similar rebound phenomenon occurs after epinephrine administration, but it is more rapid and is accounted for to a large extent by the transfer of lactic acid from the periphery to the liver. Since glucagon does not induce glycogen breakdown in muscle, the rebound which occurs in fasted animals can be explained only by gluconeogenesis. This phenomenon is not observed in alloxanized or adrenalectomized animals but reappears after insulin or cortisone treatment. These hormones therefore have a permissive action [31,56].

The intensity and duration of the hyperglycemia following a single dose of glucagon is strongly dependent on nutritional and hormonal conditions. The response is reduced by fasting [2,91,106], pancreatectomy or alloxanization [1,57]; it is normal or elevated in diabetic animals under good control with insulin [57]; and is increased by previous ACTH or cortisone treatment [67]. Obviously, the level of the glycogen stores is one of the main factors conditioning the hyperglycemic action of glucagon. Clinical

evidence as a whole is consistent with this conclusion. In diabetes in man the response to glucagon is usually of longer duration than in normal subjects, but its amplitude is quite variable [3,69,70,79,80,92]. Many authors have suggested that the endocrine state of the patient is likely to influence markedly the glycogen stores of the liver as well as the ability of the peripheral tissues to handle a sudden overload of glucose. In disease of the liver, the response to glucagon is impaired only in severe deficiencies [24,33,78,127]. In glycogen storage disease involving the liver, glucagon hyperglycemia is usually weak or absent, although in some cases it may not be very different from some normal responses [16,17,53,61,68,81,109,129]. From these numerous clinical observations it would seem that the glucagon sensitivity test does not provide much diagnostic information, except perhaps in some cases of glycogen storage disease.

*Action on Peripheral Glucose Utilization.* Widely differing conclusions regarding the peripheral action of glucagon have been reached by various investigators. A number of these conflicting results may be accounted for by impurities in the glucagon preparations used, possibly also by differences in animal species or in experimental approach. However, even taking these factors into account it is still difficult to reconcile many of the conflicting observations.

Candela (see his review [22]) has repeatedly reported that glucagon inhibits the action of insulin on rat diaphragm *in vitro*. Although this was confirmed at first [112], recent reinvestigations have given different results. According to Randle [98], insulin-free glucagon has no apparent effect on the glucose uptake of the diaphragm or on its sensitivity to insulin. Narahara and Williams [90], on the other hand, found that glucagon potentiates the action of small doses of insulin on the diaphragm, presumably by inhibiting the destruction of insulin. They did not obtain this effect with very small amounts of glucagon or when the diaphragm was incubated under conditions of maximum insulin sensitivity. Therefore, it seems unlikely that this apparent synergism of glucagon and insulin *in vitro* could explain the peripheral action of glucagon found by some authors.

In eviscerated animals no effect of glucagon has been found in the rat [72], but some depression of insulin action has been observed in rabbits [40] and dogs [93]. Experiments on intact rabbits [123] and depancreatized dogs [94] sug-

gest also an inhibition of the action of insulin on muscle. No effect was detected on the glycogen of adipose tissue in rats [57] or on the turnover rate of blood glucose in dogs [102].

Many authors have found that glucagon enhances the arteriovenous blood glucose differences (A-V), but this observation has been variously interpreted. In a careful investigation by Bondy and Cardillo [12], the increase could be ascribed entirely to the higher blood sugar level, and no effect of glucagon *per se* was detected. The same negative result was obtained by others in normal man [4] and in normal or depancreatized dogs [122]. Van Itallie initially reported an enhancement in glucose utilization over and above that induced by the hyperglycemia [128] but later could not confirm this finding [29]. However, some investigators [18,43-45,110] have concluded that glucagon stimulates glucose consumption by the periphery more than can be accounted for by equivalent hyperglycemia, and/or that it enhances the effect of insulin. It would seem, however, that very few of the published experiments are conclusive. (a) In view of the recent finding of Randle [98], the glucagon used could have been contaminated by unknown and significant amounts of insulin; (b) Even an increase in the A-V/A index, which takes into account the effect of the blood sugar level, is not unequivocal proof of a direct action of glucagon on glucose utilization in the presence of the pancreas, since it could be due to a discharge of insulin; (c) When the blood sugar is changing rapidly, A-V gradients represent not only the peripheral consumption of glucose but also the exchanges of glucose between the vascular and extravascular spaces.

It is likely that the action of glucagon on the periphery is too small to be detected with certainty by the arteriovenous difference method. On the basis of other experiments, it would seem either that glucagon has no influence on the action of insulin or only a slight effect.

*Diabetogenic Action and Insulin Antagonism.* According to current theories of the pathogenesis of diabetes, a potent hyperglycemic agent such as glucagon would be expected to be diabetogenic when given repeatedly and in large amounts. However, this property was surprisingly difficult to demonstrate. A diabetogenic action was first observed in rats, and later in rabbits, provided the animals were treated simultaneously with cortisone, ACTH or growth hormone [26,27,83]. In partially depancreatized rats some effect was



also noted [71]. In all cases, however, the symptoms of diabetes were rather mild and disappeared spontaneously during the treatment or after it was discontinued. More recently, Salter, Davidson and Best [107] have reported persistent hyperglycemia, glycosuria, negative nitrogen balance and loss of weight in rats receiving repeated injections of a glucagon suspension. The animals died after five to ten days.

A few clinical experiments have shown that continuous treatment with large doses of glucagon may have similar effects in man [11,54,66,74]. In addition, ketonemia and increased excretion of 17-hydroxysteroids were noted. It is not clear whether the adrenal stimulation thus implied is related to the improvement of joint symptoms which has been observed in some patients with rheumatoid arthritis treated with glucagon [54,66].

Two important actions of glucagon are obviously related to the diabetogenic effect described by Salter et al. Even during rather short treatments, glucagon increases nitrogen excretion [73,75,107,124]. Although it is likely that large doses of glucagon stimulate the adrenal cortex, by an unknown mechanism [30,54,66,74,79,126], the increase in nitrogen excretion occurs even in adrenalectomized rats [107]. It has also been found by the Toronto group that glucagon stimulates oxygen consumption [35]. This effect seems partially to be mediated by the adrenal medulla and to be dependent on the function of the adrenal cortex [34]. The biochemical mechanism of these two effects has not yet been investigated but it seems that they may be closely related to each other and to the gluconeogenesis induced by glucagon. The results of experiments with labelled glycine [76] would be consistent with this hypothesis. Glucagon also influences fat metabolism. It has been found to increase [54,58] or to decrease [12,75] ketonemia *in vivo*. However, *in vitro* it is definitely ketogenic [7,62], and it inhibits the synthesis of fatty acids [8,9,62,63] and cholesterol [8] by liver tissue.

It can perhaps be debated [48] whether or not glucagon has met the criteria of a true diabetogenic agent, at least until its anti-insulin effect on peripheral glucose utilization has been better evaluated. Nevertheless, glucagon acts in many respects as a powerful antagonist to insulin, as shown by experiments in which balanced doses of the two substances were given [38,124], and its effects on the liver are strikingly similar to those resulting from insulin deprivation. In fact,

its most promising clinical application appears to be as an insulin antagonist. Subcutaneous glucagon injection has been suggested as a procedure for the termination of insulin hypoglycemia [49,108]. Favorable results have been reported in one case of an insulin-producing tumor [82].

Numerous investigators who have performed experiments similar to those of the Toronto group have failed to detect any pathological consequences of repeated injections of glucagon in rats, rabbits, dogs or cats, even in large doses [48,59,60,89,103,104]. It is important to note that Salter et al. [107] used glucagon suspensions instead of solutions and therefore obtained a more continuous action. Other differences might result from varying strains of rats or from impurities contaminating the glucagon preparations. Whatever the explanation, it is likely that small doses of glucagon are not toxic. Its presence in small amounts in many commercial preparations of insulin [5,113] is probably of little importance in the treatment of most diabetics. Nevertheless, in the rabbit the subcutaneous injection of glucagon-free insulin has a more pronounced hypoglycemic effect than insulin containing glucagon [131], at least during the first hour [105]. The effects of subcutaneous injection of mixtures of insulin and glucagon have not been systematically investigated in man.

*Glucagon as a Hormone.* This important problem has been discussed extensively in recent reviews [28,37,56]. Therefore, only a few aspects will be considered here in detail.

Numerous histological investigations have shown that the  $\alpha$ -cells of the pancreas may be influenced by endocrine factors, primarily those of the anterior pituitary. Blood sugar levels and  $\alpha$ -cell morphology are sometimes modified simultaneously. In most cases the interpretation of these findings is very difficult. For example, it is difficult to determine whether lesions of the  $\alpha$ -cells are the cause or the consequence of the hypoglycemia. These investigations are nevertheless very valuable in showing some interdependence between carbohydrate metabolism and  $\alpha$ -cells, and in suggesting possible stimuli for glucagon secretion.

When  $\alpha$ -cytotoxic agents were discovered it was hoped that glucagon deficiency could be produced in animals. However, all these substances have a marked general toxicity, so that short term experiments are vitiated by pronounced side effects. Moreover, if the animals



are allowed to recover, the  $\alpha$ -cells regenerate rapidly. It would probably be difficult surgically to remove all sources of glucagon in mammals. An animal without  $\alpha$ -cells is not necessarily without glucagon, since the latter may be present in appreciable amounts outside the pancreas. Even so, it has frequently been stated that the insulin requirement in alloxan or metahypophyseal diabetes is higher than in depancreatized animals; in some experiments the difference cannot be accounted for by the exocrine secretion of the pancreas [14,21,23,25,39,87,88]. Clinical evidence argues in the same direction but is far from conclusive. Glucagon secretion in spontaneous diabetes might explain why depancreatized human subjects have rather low insulin requirements, but it is well known that many other factors could be involved. Deficient or excessive glucagon secretion has also been postulated in other disorders of carbohydrate metabolism. The application of new methods of glucagon assay may eventually provide objective support for these hypotheses.

This absence of clear-cut cases of spontaneous or experimental abnormal secretion of glucagon has been the major drawback in establishing its physiological role and status as a hormone. In consideration of the difficult interpretation of the indirect evidence mentioned, more importance has usually been attributed to direct experiments. In Young's and Foa's laboratories a hyperglycemic factor was found in the portal vein of alloxanized rats, in the pancreaticoduodenal vein of cats treated with growth hormone [13], and in the pancreatic vein of dogs during insulin hypoglycemia or after an injection of growth hormone [56]. Eser also observed the presence of a similar factor in perfusion fluid from dog pancreas [52]. However, no attempt was made to identify this substance chemically, and later investigations by Sirek, Sirek and Best [111] strongly suggest that it may have nothing in common with glucagon or the  $\alpha$ -cells. The hyperglycemic factor of the pancreaticoduodenal vein of dogs receiving growth hormone was not active in recipient animals treated with ergotamine and must therefore be a sympathicomimetic amine, since it is well known that glucagon hyperglycemia is not blocked by ergotamine. Moreover, the hyperglycemic factor was also found in the duodenal vein of depancreatized dogs.

Nevertheless, there are indications that glucagon is present in blood. Fodden and Read [55]

were the first to report that glucagon could be isolated from pancreatic blood. The amounts found by these authors were very large, since 5 per cent as much free glucagon in the plasma would have sufficed to induce marked hyperglycemia and complete glycogen breakdown in the liver. Recently, Makman, Makman and Sutherland [86] have reported much smaller amounts: 4 to 9  $\mu$ g. per 100 ml. in human plasma and 1 to 14  $\mu$ g. per 100 ml. in dog plasma. In this concentration range glucagon may be expected to have some action on the release of glucose by the liver. The purification procedure was carefully controlled, and many tests were applied to identify the substance; all the evidence suggests that it is glucagon. Tyberghein and Williams [125] have found 0.1 to 0.4  $\mu$ g. per 100 ml. in rabbit blood. Although their purification method is probably far from quantitative, it appears reproducible enough to allow comparison between blood samples. Neither of these two groups of investigators has observed any difference between pancreatic and peripheral blood. Fasting or the administration of insulin, synthalin or tolbutamide had no effect on the pancreatic or peripheral blood level [125]. Makman et al. [86] have observed large variations in the glucagon content of the peripheral blood of dogs but were unable to correlate them with physiological conditions.

These experiments must be examined with care. The half-life of injected glucagon is probably less than ten minutes [6,32]. Therefore, if the material found in plasma is the same as purified glucagon, more than 7 per cent of the circulating glucagon must be renewed per minute in order to maintain a constant level. Furthermore, if glucagon is secreted primarily by the pancreas and if the pancreaticoduodenal circulation represents, say 10 per cent of the total cardiac output, the concentration of glucagon in pancreatic blood must be at least 70 per cent higher than in peripheral blood. Since the method used by Makman et al. [86] is certainly accurate enough to detect such a difference, it follows that one of these premises must be grossly wrong. Among the many possibilities, the two most likely would be: (1) that a large part of the glucagon is secreted by the gastric mucosa; or (2) that glucagon is secreted by the  $\alpha$ -cells in a bound form with a longer biological half life than purified glucagon. Since the purification procedure applied to plasma as well as to pancreas involves drastic treatment

with acid, alcohol and ether, it is quite possible that protein which might be bound to glucagon in the native state would be denatured under these conditions.

Despite lack of definite proof of the hormonal nature of glucagon, it is tempting to speculate about its role. As we do not know what factors stimulate glucagon secretion, teleological considerations based on its known properties are the starting point of these hypotheses.

The remarkable position of glucagon in the system of insulin antagonists has frequently been noted. It shares with epinephrine a rapid action of short duration, in contrast to the slower influence of the pituitary and adrenal systems. However glucagon does not exhibit clear-cut peripheral actions. The secretion of glucagon into the portal circulation would also tend to restrict its effects to the liver. Therefore, if a hormonal agent is required to promote the transfer of glucose from the liver to the periphery, glucagon seems well adapted to that function. It should be pointed out that epinephrine could not perform this function as well. Epinephrine inhibits peripheral glucose utilization and stimulates glycogen breakdown with the formation of lactic acid, which is partly converted into glycogen by the liver. Thus one of the end results of epinephrine action is a net transfer of carbohydrate from the periphery to the liver. Speculations about the role of glucagon have usually been limited to its effects on the blood sugar and the liver glycogen. However, the other properties of the hypothetical hormone should be kept in mind. It may turn out that its actions on lipid and protein metabolism, or its action on the kidney [46,115] are physiologically as important as its direct effect on carbohydrate metabolism in the liver.

*Mechanism of Action of Glucagon.* Recent progress in this field has been so rapid that glucagon and epinephrine may prove to be the first hormones with known biochemical mechanisms of action. After glucagon was rediscovered, it was rapidly established by Sutherland and Cori [116,117] that glucagon and epinephrine enhance glucose production by stimulating the phosphorylytic pathway of glycogen breakdown. Phosphorylase is the rate-limiting enzyme of the glycogenolytic sequence, at least *in vitro*. The two hormonal agents were found to activate liver phosphorylase *in vivo* and *in vitro*. Further investigations by Sutherland and his collaborators [97,121,134,135] showed that liver phosphorylase

(LP) is a phosphoprotein which is transformed by dephosphorylation into an inactive form (dephospho-LP). This reaction is catalysed by a phosphoproteinphosphatase (LP-phosphatase). The inactive LP can be reactivated by rephosphorylation with ATP and a specific kinase. The level of active LP in the liver is thus the resultant of two antagonistic systems. There is good evidence that the turnover of LP to and from dephospho-LP is rapid. Under basal conditions the level of LP is low, and most of the enzyme is present in the inactive form. Glucagon and epinephrine rapidly shift the dynamic equilibrium of the system toward a high level of active LP. (Fig. 1.)

When a response to epinephrine and glucagon was obtained in a cell-free liver homogenate, it was shown that the hormones initiate a series of reactions [96,120]. A particulate component of the liver cell is stimulated to synthesize a special nucleotide, 3',5'-adenosine monophosphate (3',5'-AMP), from adenosine triphosphate (ATP). The 3',5'-AMP induces faster LP formation (or slower LP breakdown) in the soluble fraction of the cytoplasm and therefore displaces the dynamic equilibrium toward a higher level of active LP. Some important questions are still unanswered. The cytological nature of the particles, the way they are stimulated by the hormones, and the mechanism by which the nucleotide acts on the phosphorylase-regulating system are still obscure.

There is little doubt that the mechanism brought to light *in vitro* also is operative *in vivo*. Moreover, a similar mechanism has been found in other tissues. Epinephrine induces very rapid glycogen breakdown in muscle and in heart. Almost certainly these two tissues react to epinephrine by first forming 3',5'-AMP which in turn elicits phosphorylase reactivation [95]. The most surprising discovery in this field was that ACTH probably influences the adrenal cortex by the same mechanism. The following sequence of reactions has been shown to occur in homogenates of this tissue [50,64,65]: the particles are stimulated by ACTH to form increased amounts of 3',5'-AMP; the latter induces phosphorylase reactivation; glycogen is broken down to hexose phosphates; more glucose-6-phosphate is available for the pentose phosphate pathway, and therefore increased amounts of reduced triphosphopyridine nucleotide are formed; a larger supply of the reduced coenzyme results in a greater formation of corticoids.

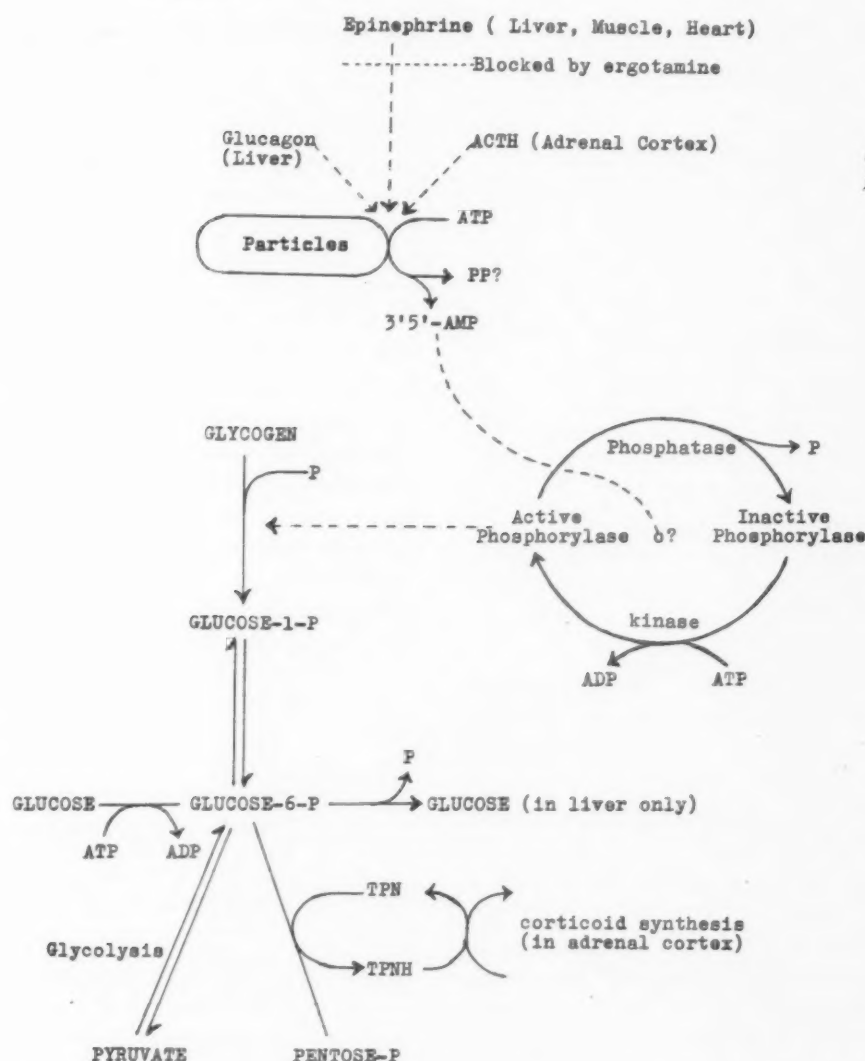


FIG. 1. Mechanism of action of glucagon, epinephrine and ACTH: PP, pyrophosphate; 3',5'-AMP, 3',5'-adenosine monophosphate; TPN and TPNH, oxidized and reduced triphosphopyridine nucleotide.

To date, four tissues are thus known to react specifically to one hormone (or to two in the case of the liver) by forming 3',5'-AMP. In each case phosphorylase reactivation results. But the ultimate result is quite different: in liver, glucose output; in muscle and heart, glycolysis and lactic acid production; in the adrenal cortex, corticoid synthesis. In addition, the nucleotide may influence other enzyme systems. It has already been found to exert a ketogenic effect on isolated liver slices and thus appears to be involved also in this action of glucagon and epinephrine on lipid metabolism [7].

In the preceding schema it has been assumed that glycogen breakdown would necessarily result from greater phosphorylase activity. *In vitro*, the phosphorylase reaction is easily re-

versible. Its direction *in vivo* would therefore depend on the local concentrations of glucose-1-phosphate (G-1-P) and phosphate (P). Since the discovery of phosphorylase by the Coris, it had been assumed that this enzyme is involved both in the synthesis and in the breakdown of glycogen. However, tissue analyses have shown that the concentrations of G-1-P and P are probably such that breakdown would always be favored. Moreover, it has been noted frequently that glycogen synthesis occurs in liver or muscle only when most of the phosphorylase is in the inactive form. A high level of active phosphorylase is always associated with glycogen breakdown. This suggests that, in the intact cell, phosphorylase catalyses only glycogenolysis and that glycogen synthesis must occur by a



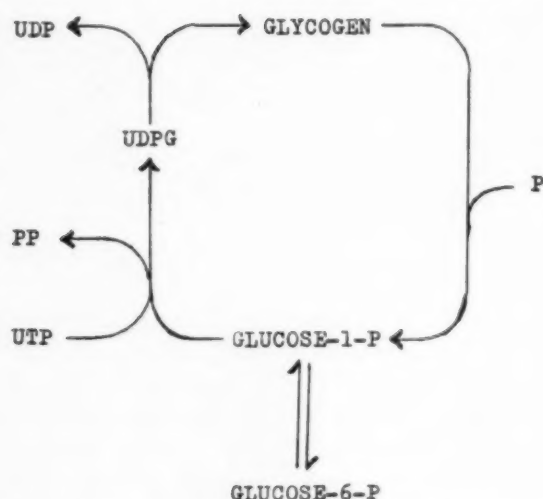


FIG. 2. Hypothetical pathways for the synthesis and breakdown of glycogen: PP, pyrophosphatase; UTP, uridine-triphosphate; UDP, uridine diphosphate; UDPG, uridine diphosphoglucose.

different pathway. An enzyme involving uridine diphosphate glucose (UDPG) as the glucosyl donor has indeed been found recently [84,130]. (Fig. 2.) UDPG is easily formed from uridine triphosphate (UTP) and glucose-1-phosphate. The reactions are probably reversible, but glycogen synthesis can proceed if uridine diphosphate (UDP) and pyrophosphate (PP) are continuously removed. If this new pathway of glycogen synthesis is confirmed by further investigation, the glycogenolytic action of glucagon, epinephrine and ACTH will be satisfactorily explained by the activation of phosphorylase.

#### SUMMARY

Crystalline glucagon as well as other purified preparations are available for study. However, the results obtained with such preparations should still be considered with some caution, in view of the possibility of their contamination with insulin or other active proteins.

The  $\alpha$ -cells are the most probable site of formation of glucagon in the pancreas, but its presence in the gastric mucosa of some species should not be overlooked.

The hyperglycemic effect of glucagon results from the breakdown of glycogen in the liver. The level of the glycogen stores is the main factor determining the hyperglycemic response to glucagon. The effect on peripheral glucose utilization is doubtful and probably of little importance.

The effects of glucagon on protein and lipid metabolism and on oxygen consumption suggest

a general catabolic action, strongly reminiscent of the consequences of insulin deprivation on the liver. All the catabolic responses to glucagon appear to contribute to the induction of diabetic symptoms when large doses are given.

The hormonal status of glucagon is still uncertain. Nevertheless, recent experiments make it appear likely that it is present in plasma. Technical improvements give hope that its secretion by the pancreas or other tissues soon will be established, and that its role in physiology and pathology will be clarified.

The biochemical mechanism of action of glucagon in liver is complex. It involves the formation of 3',5'-AMP which in turn influences the enzyme system controlling the level of active phosphorylase. A basically similar mechanism apparently is involved in some actions of epinephrine on heart and muscle, and of ACTH on the adrenal cortex.

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# Galactose Metabolism and Galactosemia\*

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THE metabolism of galactose has received considerable attention in recent years as further information concerning its biochemical pathways and reactions has become apparent. Much of the newer knowledge of galactose utilization has resulted from the discovery of the role of the uridine nucleotides in carbohydrate metabolism and from the realization of the important function of these compounds in the interconversion of galactose and glucose. Most of the advances in this area of metabolism can be attributed directly to the significant observations of Leloir [1,2] and Kalckar [3,4].

Galactose is a six-carbon sugar or hexose which is similar to glucose in structure except for the orientation of the hydrogen and hydroxyl groups about the fourth carbon atom. (Fig. 1.) It is an important source of body energy, especially in the early years of life when milk is the principal dietary constituent. Galactose is most abundant in milk in which it appears in the form of the disaccharide, lactose. The latter is hydrolyzed enzymatically in the intestine to yield its two component sugars, galactose and glucose (Fig. 1), which are then absorbed and transported to the liver.

Galactose also forms an integral part of a number of vital tissue compounds. One of the most important of these is in the brain where galactose is found in the important group of lipids known as the cerebroside. It is an important constituent of some mucopolysaccharides of connective tissue, especially of the chondroitin sulfate in cartilage. Galactose also occurs in the mammary gland in the form of lactose which is synthesized there under the influence of the lactogenic hormone. It is important to point out that current evidence indicates that the galactose found in these tissues and substances is *not* derived directly from the dietary or ingested galactose. Rather the major portion of the galactose in

these compounds is derived from glucose and other precursors. It follows, therefore, that individuals may be able to synthesize galactose-containing compounds even though no galactose is ingested. This observation is relevant to the problem of galactosemia and its management with galactose-free diets.

Clinically, several diseases are associated with derangements of galactose metabolism. These are often reflected by impairment of the galactose tolerance test, as in liver disease for example [5]. In thyrotoxicosis up to 75 per cent of patients show an abnormal tolerance to orally administered galactose but the reaction to the intravenous test is normal [6]; this anomaly is due to enhanced intestinal absorption of galactose in this disease. By far the most pronounced disturbance of galactose metabolism, however, occurs in galactosemia.

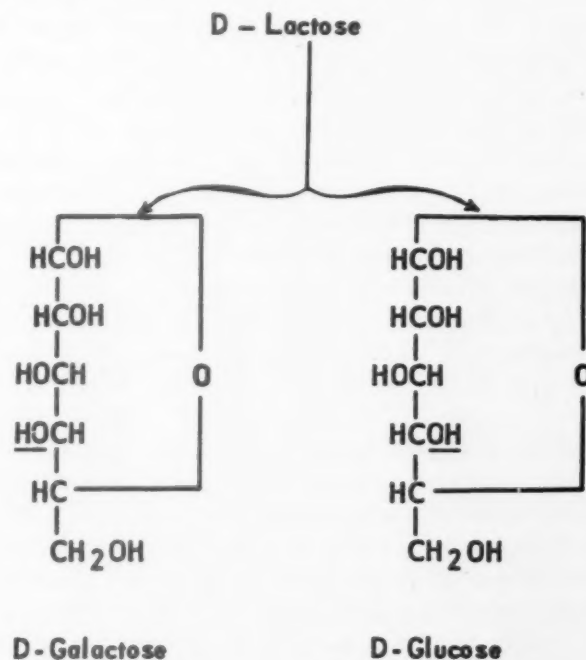


Fig. 1. Chemical structure of galactose and glucose.

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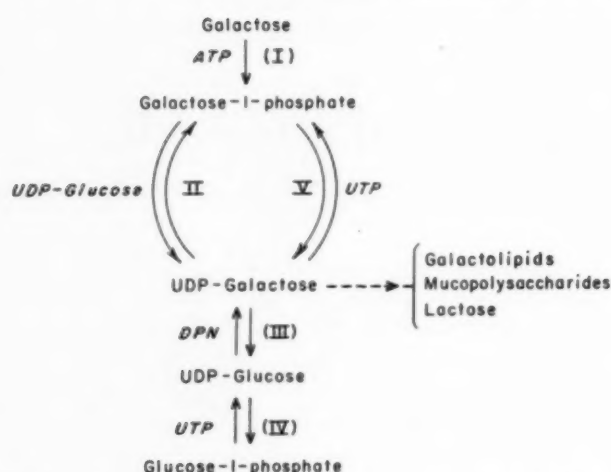
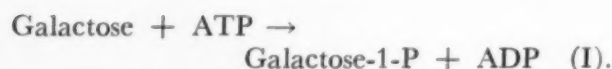


FIG. 2. A schematic representation of the reactions and interactions important in the metabolism of galactose. Roman numerals are the same as those used in the text to refer to the specific enzymes catalyzing the various reactions. Abbreviations include ATP, adenosine triphosphate; UDP-glucose, uridine diphosphate glucose; UDP-galactose, uridine diphosphate galactose; UTP, uridine triphosphate.

#### BIOCHEMICAL ASPECTS OF GALACTOSE METABOLISM

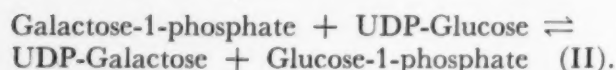
Galactose, after its absorption from the intestinal tract, is transported to the liver where it is converted to a large extent into glucose derivatives and thus enters the general "glucose pool." At least five enzymatic reactions are important in this galactose-glucose interconversion. It is desirable to review these reactions in some detail.

**Galactokinase.** This enzyme phosphorylates galactose to  $\alpha$ -galactose-1-phosphate. This reaction requires adenosine triphosphate (ATP) in a reaction analogous to that catalyzed by the enzyme hexokinase.



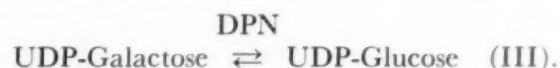
This reaction is not reversible. Adenosine diphosphate is also formed. The enzyme is known to be present in liver, brain and red cells.

**Galactose-1-Phosphate Uridyl Transferase.** The conversion of galactose-1-phosphate to glucose-1-phosphate is now known to occur by means of a reaction which involves a specific cofactor, namely the nucleotide, uridine diphosphoglucose (UDP-Glucose).



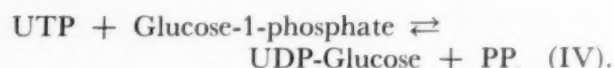
In this reaction galactose-1-phosphate is transferred to the uridine nucleotide to yield uridine diphosphogalactose (UDP-Galactose) and glucose-1-phosphate is in turn derived from UDP-Glucose. The enzyme catalyzing this reaction is often abbreviated as Gal-1-P uridyl transferase and has also been referred to as P-Gal transferase or Gal-1-P transuridylyase. This enzyme is of prime importance in the utilization of galactose-1-phosphate and for its incorporation into UDP-Galactose. It should be noted that another reaction, to be described (V), can also serve to incorporate galactose-1-phosphate into UDP-Galactose, but does so to a lesser extent. Figure 2 illustrates how UDP-Galactose is involved in the synthesis of such galactose-containing compounds as galactolipids, mucopolysaccharides and lactose. Galactose-1-phosphate uridyl transferase is the enzyme which is congenitally absent and deficient in galactosemia.

**UDP-Galactose-4-Epimerase.** In a third reaction the two uridine nucleotides are directly interconverted. The enzyme catalyzing this reaction was previously called "galactowaldenase." However, more recent data show a requirement for diphosphopyridine nucleotide (DPN), hence the enzyme has been called an "epimerase." It is believed that in this reaction two hydrogens are removed from the fourth carbon of the galactose of the nucleotide, and then added back but in the opposite direction, so that the glucose epimer is formed [7].



It is important to note that this key enzyme catalyzes a reversible reaction and that therefore UDP-Galactose can be formed from UDP-Glucose. The latter is in turn synthesized from glucose-1-phosphate by the next reaction (IV) to be described. This again demonstrates why dietary galactose is not essential for UDP-Galactose formation.

**UDP-Glucose Pyrophosphorylase.** This enzyme is not directly involved in galactose-glucose interconversion but is a means of synthesizing the nucleotide UDP-Glucose involved in the preceding two reactions.



The reaction is reversible and requires uridine triphosphate (UTP), pyrophosphate (PP) being

TABLE I  
CLINICAL AND LABORATORY FINDINGS  
IN GALACTOSEMIA

Clinical Features	Laboratory Findings
1. Nutritional failure	Elevated blood galactose
2. Hepatosplenomegaly, jaundice, cirrhosis	Reduced galactose tolerance
3. Cataracts	Deficient erythrocyte galactose-1-phosphate uridyl transferase
4. Mental retardation	Urine: galactose, amino acids, albumin

formed as a product of the reaction. The enzyme is abundant in liver, mammary gland, muscle and red cells.

*UDP-Galactose Pyrophosphorylase.* Recent observations [8,9] indicate that mammalian liver also contains a pyrophosphorylase which catalyzes the following reaction:



The activity of this enzyme in neonatal liver tissue is very weak but increases with age. Its activity (per mg. protein) in adult liver is about one-sixth that of Gal-1-P uridyl transferase. It is evident that this enzyme provides an additional pathway for galactose-1-phosphate utilization and incorporation into UDP-Galactose. The enzyme has been found in liver and brain but has not been detected in erythrocytes.

Knowledge of these reactions and their interrelationships, as shown in Figure 2, now provides a much better understanding of the normal metabolism of galactose, as to its utilization and synthesis, and also provides a sound basis for studying and elucidating the fundamental defect in galactosemia.

#### CLINICAL ASPECTS OF GALACTOSEMIA

Galactosemia is a congenital and hereditary disorder characterized by a block in the metabolism of ingested galactose or galactose-containing substances such as the lactose in milk. More than forty cases have been reported in the literature [10-12] and many more unreported cases have been discovered in the course of investigations of this disease.

*Clinical Manifestations.* The symptoms and signs which occur in galactosemia are directly related to the ingestion of galactose-containing foods and usually manifest themselves in the neonatal period. The main clinical as well as

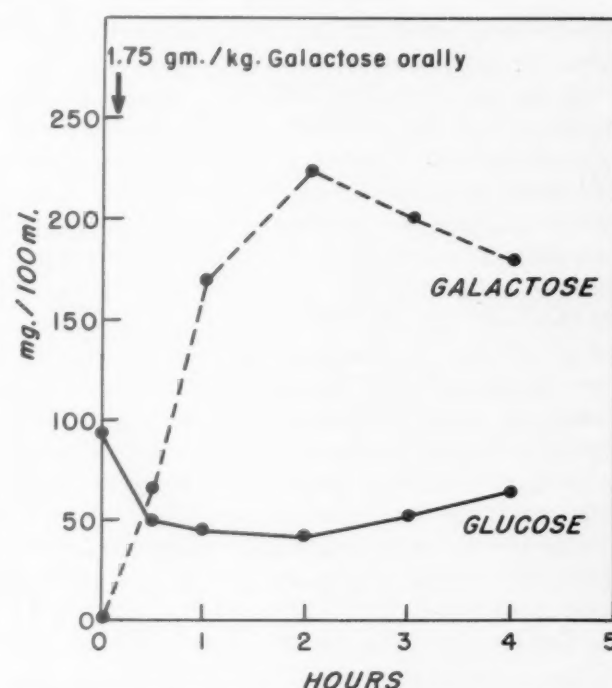


FIG. 3. The effect of an oral galactose tolerance test on the blood glucose and galactose levels in an eight year old patient with galactosemia.

laboratory features are listed in Table I. The classic cases display an impairment of nutrition followed by a triad of symptoms characterized by hepatosplenomegaly, cataract formation and mental retardation.

Affected infants usually appear normal at birth but after several days or weeks of milk ingestion vomiting and diarrhea often develop, which may then be followed by dehydration. The poor caloric intake and gastrointestinal losses contribute to impaired nutrition and growth. Jaundice is a common occurrence due to the parenchymal involvement of the liver. Initially the liver shows fatty infiltration which readily proceeds to a cirrhosis. If the clinical condition extends beyond a period of four to eight weeks, one can usually detect evidence of cataract formation and mental retardation. The infants usually are reluctant to eat, and may show lethargy and hypotonia. Depression of blood glucose levels during the ingestion of milk or galactose is not unusual, and typical hypoglycemic symptoms may occur. One has therefore the paradoxical situation of an elevated blood galactose level but a blood glucose level which is below normal. This may also occur during a galactose tolerance test. (Fig. 3.) On the basis of the experimental observations of Foa [13] the lowered blood glucose level may be due to

the stimulation of insulin release from the pancreas by the elevated blood galactose level.

It should be emphasized that in addition to patients with the classic features described, there are others with less pronounced manifestations in whom disturbances of growth, liver function or the central nervous system may not attract attention for weeks or months. Frequently these patients are the ones who have shown "milk intolerance" in the early days of life; presumably, because of a reduced milk intake and the use of milk substitutes severe clinical manifestations do not develop in these patients. It is readily apparent that the diagnosis may be missed in such patients on clinical grounds alone. Patients in mental institutions who have recently been discovered by means of the red cell enzyme test [14] to have galactosemia probably fall into this latter group.

The earliest clue to the diagnosis of galactosemia usually comes from the finding of a reducing sugar in the urine, which when analyzed by the various techniques to be described is identified as galactose. It is important to emphasize that the galactosuria may be inconstant, for it is obviously dependent on the ingestion of galactose and this may be minimal if the patient has been vomiting. Furthermore, errors have often been made when the patient, upon admission to the hospital, is given parenteral fluid therapy, and an initial urinalysis containing reducing substance is assumed to be due to the glucose intravenously administered.

In addition to galactosuria, these patients often show variable degrees of albuminuria and aminoaciduria. The amino acid pattern has been essentially similar in all cases, with predominance of the neutral, aliphatic chain type, i.e., serine, glycine, alanine, threonine, glutamine and valine. In addition, small quantities of phenylalanine, lysine, cystine, glutamic acid, methyl histidine, tyrosine and aminoisobutyric acids have been detected in the urine of these patients [15,16].

*Therapy and Subsequent Course.* If significant milk ingestion persists in patients with galactosemia, death usually results. On the other hand, if the diagnosis is made before the disease is too far advanced, one of the striking features is that all the symptoms and signs may regress and even disappear completely when these patients are given galactose-free diets. Rapid weight gain ensues, with cessation of nausea, vomiting and diarrhea. The liver and spleen

often return to normal size [10]. The major symptom which usually shows no improvement is the mental retardation. It has been observed by most investigators that damage to the central nervous system may not improve despite the most rigid adherence to a galactose-free diet. For this reason early diagnosis and prompt institution of therapy are extremely important.

Galactose-free diets can readily be instituted in infancy by resorting to commercially available milk substitutes such as Nutramigen® and Dextri-Maltose®. It has been amply demonstrated that these diets are compatible with normal development and growth even though, as Holzel et al. [17] point out, some may contain traces of galactose. Most of the patients that we have seen have been maintained on these commercial preparations and have done well. In none have mental retardation, cataract formation, liver disease, or any symptoms occurred as a result of these diets. We therefore do not share the view of Holzel et al. that the traces of galactose in these commercial milk substitutes are harmful and that more strict artificial diets are essential. We have, indeed, considered the gastrointestinal disturbances that may accompany the lactose-free diet of Holzel, Komrower and Schwarz [18] to be a drawback in the very young infant.

Although Platt [19] considers that the ingestion of galactose may be important for normal development and that galactose is poorly synthesized in the body, there is at present no definite clinical evidence that central nervous system function, or somatic development in general, is impaired by the prolonged use of galactose-free diets. There is ample evidence, as will be indicated, that patients with galactosemia possess the enzyme, UDP-Galactose-4-epimerase, which allows for endogenous synthesis of galactose from glucose and hence permits the formation of galactolipid in the central nervous system [20] in the absence of dietary galactose.

#### ENZYMATIC DEFECT IN GALACTOSEMIA

Although there has been much speculation since 1935 concerning the basic lesion in galactosemia, no definitive answer could be forthcoming until more detailed information concerning the biochemistry of galactose metabolism and the galactose-glucose interconversion was available. The first important observation which pointed toward the underlying lesion was that of Schwarz et al. [27] who showed that red cells



were able to metabolize galactose but that galactosemic red cells were abnormal in this respect. These workers noted that erythrocytes of patients with galactosemia accumulate galactose-1-phosphate after milk or galactose is ingested. These observations were confirmed by us in *in vitro* experiments in which it was shown that galactosemic red cells, incubated in a galactose-containing medium, showed significant amounts of galactose-1-phosphate in contrast to normal red cells similarly incubated [22]. Both of these observations strongly suggested that the defect in galactosemia might be due to a block in the conversion of galactose-1-phosphate to glucose-1-phosphate, and that such a block might reasonably involve the enzyme Gal-1-P uridyl transferase. Although the occurrence of UDP-Galactose pyrophosphorylase in mammalian tissues was not appreciated at the time of these initial observations, the fact that this enzyme is not normally present in red cells would still have focused attention on the deficiency of Gal-1-P uridyl transferase as a plausible explanation for the *in vivo* and *in vitro* accumulation of galactose-1-phosphate.

Specific studies on red cells aimed at elucidating the site of the abnormal galactose metabolism have disclosed that normal red cells contain the enzymes catalyzing reactions I through IV. In contrast, galactosemic red cells have been shown specifically to be lacking in enzyme II, Gal-1-P uridyl transferase [22,23]. This observation has been confirmed in all cases of galactosemia studied thus far. It has been shown that galactosemic red cells neither lack any cofactors nor contain any demonstrable inhibitors to account for the lack of transferase activity. It was initially reasoned that the absence of the enzyme in the erythrocytes might simply have been due to an adaptive phenomenon; that is, since many of the galactosemic patients tested had not taken galactose for extended periods of time, the red cells might gradually have lost their transferase activity. However, when erythrocytes of patients on galactose-free diets for as long as eighteen months were examined they were found to be normal with respect to all three enzymes [22]. Also it has been shown that the transferase deficiency is present at birth, since it has been demonstrated that the transferase is absent from the cord blood of infants afflicted with the disease [24].

As would be expected, the liver also is deficient in this enzyme, as demonstrated in the liver tis-

sue obtained from an infant and an adult with this disorder [24]. However, the erythrocytes of the blood are a more convenient means of studying the enzymatic pattern of the patient's somatic cells, and are employed in an adaptation of the enzymatic assay for Gal-1-P uridyl transferase for use as a specific diagnostic test for galactosemia [25].

#### TESTS IN THE DIAGNOSIS OF GALACTOSEMIA

*Characterization of the Nature of the Mellituria.* When one finds a reducing sugar in an infant, with or without the classic features of galactosemia described, it is imperative to establish the identity of the sugar. Recently this has been greatly facilitated by methods utilizing the enzyme glucose oxidase. Commercial test preparations containing glucose oxidase, such as Test-Tape® and Clinistix®, give a positive reaction only in the presence of glucose; thus non-glucose sugars can readily be suspected. A recently reported method [26] using a microbial galactose oxidase is promising since it will permit rapid and definitive identification of galactose in biologic fluids. In addition to the usual chemical methods for identifying galactose (e.g., oxidation of the sugar to mucic acid), paper chromatography has been employed to assist in the diagnosis [27].

*Galactose Tolerance Test.* In the past it has been customary to confirm the diagnosis of galactosemia by means of the oral or intravenous galactose tolerance test [10,12]. As already mentioned, this test is not without hazard, especially in an infant in whom there is marked impairment of galactose utilization. In these patients hypoglycemia develops readily during the course of the test (Fig. 3) and serious reactions such as convulsions may occur. For this reason and the fact that infants with galactosemia preferably should not be exposed to galactose if at all possible, one should try to avoid the galactose tolerance test, especially since it is now possible to make the diagnosis by the other methods (to be described) which do not jeopardize the health of the infant.

*Erythrocyte Assay for Galactose-1-Phosphate Uridyl Transferase.* As a result of the observations that circulating erythrocytes normally contain abundant amounts of Gal-1-P uridyl transferase, a test has been developed which is based on the absence of this enzyme in red cells of patients with galactosemia. This method, the details of which have been outlined elsewhere [25], is both

sensitive and specific, and no false positives have been encountered. Patients with diseases such as hepatitis or cirrhosis in whom galactose tolerance is abnormal do not demonstrate a defect of transferase activity in their red cells. Errors may occur, however, if a galactosemic patient has been transfused with normal blood within a period of three months prior to the assay. In such a situation the transferase content of the transfused normal cells may give a false result [24].

This enzymatic method has a number of advantages: (1) it is quickly performed and the result can be obtained in several hours; (2) it is possible to establish the diagnosis at birth on umbilical cord blood, a feature desirable if the newborn infant is a member of a galactosemic family; (3) the hazards of galactose administration are avoided; (4) the test is specific.

*Galactose-1-Phosphate Accumulation in Erythrocytes.* Schwarz, Holzel and Komrower have advocated the use of an *in vitro* test which is based on the accumulation of galactose-1-phosphate in galactosemic red cells [28]. In this test the red cells are incubated with galactose, subsequent to which phosphorylated derivatives are isolated as their barium salts. The sugar phosphates are then hydrolyzed and the free sugars separated by paper chromatography. The galactose is estimated by comparison with known quantities chromatographed simultaneously. The main disadvantage of this test is that it requires two to three days to carry out. However, as with the enzymatic procedure, this test can be performed on cord blood and the diagnosis can therefore be made several days after birth.

#### ADDITIONAL ASPECTS OF GALACTOSEMIA AND GALACTOSE METABOLISM

*Possible Toxic Factors in Galactosemia.* Animal and bacterial studies have shed considerable light on the factors which may account for the pronounced physiologic disturbances that occur in galactosemia. It has been observed repeatedly that in pigeons or chicks placed on diets containing 30 per cent galactose a typical quivering syndrome and other neurologic manifestations, such as ataxia, often develop [29]. In rats on similar diets cataracts uniformly develop within a period of two to three weeks. Studies of the affected lenticular tissue of these animals have shown that they contain significant quantities of galactose-1-phosphate [30], just as in the case of galactosemic red cells. In microbiologic studies Kurahashi and Wahba [37] have described a mutant of *Escherichia coli* which is deficient in

Gal-1-P uridyl transferase. These organisms grow normally on a glucose medium but when galactose is added growth is impaired and the bacteria accumulate increasing quantities of galactose-1-phosphate. It is thus tempting to speculate that it is not the galactose itself which is directly toxic but rather its phosphorylated derivative. It is conceivable that this toxicity of galactose-1-phosphate may be due to its inhibition of one or more enzyme systems. In this connection it has recently been observed *in vitro* that galactose-1-phosphate can inhibit the enzyme phosphoglucomutase, provided that the co-factor, glucose-1,6-diphosphate, is not present in excess [32,33]. Whether or not this inhibition is of any relevance to the clinical manifestations is difficult to evaluate at this time.

It is conceivable that cellular depletion of ATP might be a factor in the toxic manifestations. This could result from the fact that the cells might lose a significant amount of their ATP in forming galactose-1-phosphate, which accumulates and is not readily metabolized. In a recent study, Penington and Prankerd [34] have actually observed a lowering of erythrocyte ATP levels when a galactosemic patient was given a lactose diet.

Hypoglycemia, which may occur in galactosemia, has also been considered by some to be causally related to the clinical manifestations of the disease, especially in regard to the mental retardation. However, it should be recalled that the symptoms and signs found in galactosemia, notably the changes in the lenticular and central nervous system, do not occur in such conditions as von Gierke's disease in which episodes of hypoglycemia are pronounced and severe.

*Possible Alternate Pathways of Galactose Metabolism in Galactosemia.* In spite of the demonstrable enzymatic lesion and defect in galactosemia, it is apparent that some utilization of exogenous galactose occurs. In some of the earlier clinical observations it was noted that patients with this disease show improvement in their galactose tolerance test as they age [10,12]. In fact, adult patients with galactosemia may ingest varying quantities of galactose without experiencing any significant side effects. When one administers galactose to a galactosemic patient, from 30 to 80 per cent of the ingested galactose is retained and not accounted for by the urinary excretion of the sugar. From previous studies [21,22,35] one would expect that much of the retained galactose would be in the form of galactose-1-phosphate. Studies performed with C<sup>14</sup>-labeled

TABLE II  
METABOLISM OF C<sup>14</sup>-GALACTOSE IN AN ADULT PATIENT  
WITH GALACTOSEMIA\*

Determination	Amount (mg.)
Galactose in urine.....	700
Galactose metabolized to glucosiduronic acid.	30
Galactose accumulating as cellular galactose (galactose-1-phosphate, etc.).....	50-100†

\* Values after the intravenous infusion of 1 gm. of galactose (containing 5 microcuries of galactose-1-C<sup>14</sup>) to a twenty-four year old man with galactosemia; 1 gm. of menthol was given orally.

† All tissues with the exception of muscle and bone. (From: EISENBERG, F., JR., ISSELBACHER, K. J. and KALCKAR, H. M. *Science*, 125: 116, 1957 [36].)

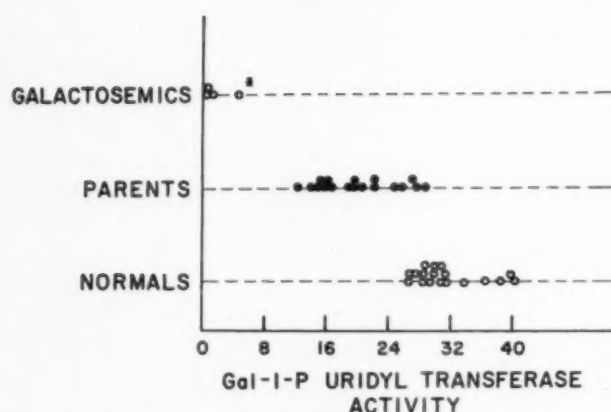
galactose in a twenty-four year old man with galactosemia have permitted a more definitive analysis of the extent of galactose utilization in this disease [36]. The results are reproduced in Table II. It is apparent that 3 per cent of the administered galactose could be accounted for in the urine as a glucosiduronic acid. In view of our knowledge of the pathways of glucuronide and glucosiduronic acid formation, the data indicate that at least 3 per cent of the administered galactose must have been converted to glucose derivatives. The same experiment in a normal person resulted in no significantly greater utilization of galactose for glucosiduronic acid formation. These experiments then confirm the suspicion that some galactose utilization does occur in galactosemia, at least in the adult patient. This would suggest that either the defect of Gal-1-P uridyl transferase is incomplete or that there is another pathway for the utilization of galactose-1-phosphate. Since the enzymatic defect in the erythrocytes is virtually complete, the possibility of another pathway of galactose metabolism has to be considered. It is evident from the biochemical reactions already reviewed, and shown in Figure 2, that the enzyme UDP-Galactose pyrophosphorylase could function in such a manner and allow for the further metabolism of galactose-1-phosphate and its incorporation into UDP-Galactose. The theory that this enzyme accounts for the utilization of galactose in the adult galactosemic patient is appealing because this enzyme has been observed to be weak in fetal and neonatal tissue but to increase in activity with the age of the organism [8]. It seems reasonable to postulate, therefore, that patients with

galactosemia have their most pronounced symptoms at birth because at this time Gal-1-P uridyl transferase is absent and in addition UDP-Galactose pyrophosphorylase activity is weak. The subsequent increase in activity of the latter enzyme with age could then account for the improvement in galactose tolerance and utilization which occurs as the patient matures.

*Factors Influencing Galactose Metabolism.* Studies by Tygstrup et al. [37] on the use of galactose for hepatic blood flow measurements have shown a marked depressant effect of ethanol on the ability of the liver to remove galactose from the blood stream. The site of this effect is not yet known but it may be the same as that which interferes with galactose utilization in liver disease. Recent observations indicate that certain steroids have a striking effect on galactose oxidation. Pesch and Topper, using liver slices, have shown that steroids such as progesterone and testosterone cause a greater than threefold stimulation of galactose-1-C<sup>14</sup> oxidation, as measured by C<sup>14</sup>O<sub>2</sub> formation [38]. A similar steroid effect was not demonstrable with glucose-1-C<sup>14</sup> as the substrate. A more recent observation by these investigators [39] indicates that progesterone also stimulated the oxidation of galactose-1-C<sup>14</sup> to C<sup>14</sup>O<sub>2</sub> in a young patient with galactosemia. These findings suggest that steroids such as progesterone may be of therapeutic value in the treatment of galactosemia, especially in the neonatal period when toxic symptoms are pronounced. One again wonders whether or not these steroids stimulate the alternate (pyrophosphorylase, V) pathway of galactose metabolism.

*Hereditary Aspects of Galactosemia.* The frequent occurrence of galactosemia in siblings and among the offspring of consanguineous matings, together with its equal incidence in both sexes suggests that it is transmitted by a single autosomal recessive gene. Holzel and Komrower [40] have investigated the genetics of galactosemia using the oral galactose tolerance test. They found mild impairment of galactose tolerance in some relatives but were unable to show abnormal tolerance in *both* parents. It is difficult to reconcile this observation with the concept that galactosemia is a simple recessive disease in which *both* parents should be heterozygous. However, it is apparent that this test is not sufficiently sensitive for definitive conclusions concerning the genetic mode of inheritance in galactosemia. The same limitation applies to the use of the Gal-1-P uridyl transferase assay for genetic





\*Represents a patient with galactosemia after transfusion with normal blood.

FIG. 4. A comparison of galactose-1-phosphate uridyl transferase activities in patients with galactosemia, their parents, and normal control subjects. Activity is expressed as microliters of oxygen uptake per hour for 0.3 ml. (From: KIRKMAN, H. N. and BYNUM, E. [42]).

studies. Although this test is reliable and specific, the kinetics of the method are such as to make it difficult to assay quantitatively intermediate amounts of the enzyme. Nevertheless, Hsia et al. have used the transferase assay in genetic studies and, in a preliminary report of their observations on five galactosemic families, described decreased Gal-1-P uridyl transferase activity in heterozygotes [41]. It should be noted that the difference in values between their normal control subjects ( $4.5 \pm 0.47$  units/gm. hemoglobin) and the heterozygotes ( $3.3 \pm 0.36$ ) are such as to make it difficult to interpret the results in any individual case.

A method which is much more suitable for genetic studies has recently been developed by Kirkman and Bynum [42]. This assay is a manometric one using hemolysates of human erythrocytes, and is carried out in such a manner that zero-order kinetics obtain. In a study of eighteen parents of galactosemic patients, it was found that all parents possessed enzymatic activity below the normal mean. Thirteen of the eighteen parents had activities which were more than 2 standard deviations below the normal mean. Some of their data are reproduced in Figure 4. It is apparent that parents, as well as normal persons, vary somewhat in the Gal-1-P uridyl transferase activity demonstrated, and that the values in parents and normal control subjects overlap slightly. It is likely, as these authors point out, that the variation observed in the enzymatic activity represents a combination of environmental factors, laboratory errors and (possibly) other genes, but that nevertheless all

parents are heterozygous and possess diminished transferase activity.

It is still not known whether these heterozygous individuals have a reduced quantity of unaltered and structurally normal enzyme or whether the lowered activity which they demonstrate is the result of *hybrid* gene products. These and other considerations as they apply to "biochemical genetics" have recently been admirably reviewed by Kalckar [43].

#### SUMMARY

1. Galactose forms an integral part of a number of biologically important compounds, such as cerebrosides, mucopolysaccharides and lactose. Most of the galactose present in these substances is not derived directly from ingested galactose but rather is synthesized from glucose and other precursors.

2. Ingested galactose after absorption is transported to the liver, converted to glucose derivatives, and then enters the general "glucose pool." The enzymatic reactions involved in galactose-glucose interconversion are reviewed.

3. Impairment of galactose utilization occurs in liver disease, thyrotoxicosis, after ingestion of ethanol, and in galactosemia.

4. Galactosemia is a congenital disorder of galactose metabolism which is transmitted by a single recessive gene and is due to the deficiency of the enzyme, galactose-1-phosphate uridyl transferase. The clinical manifestations consist of nutritional failure, hepatosplenomegaly, cataracts and mental retardation. The urine contains galactose, varying types and amounts of aminoacids, and albumin. Most of the clinical manifestations, except the mental changes, usually improve when galactose is removed from the diet. The toxic manifestations may be related to the accumulation of galactose-1-phosphate in the tissues. The presence of galactose-1-phosphate uridyl transferase in normal erythrocytes and its absence in galactosemic cells has provided the basis for a rapid and specific diagnostic test for the disease.

5. Some utilization of galactose occurs in patients with galactosemia and increases as they mature. This may occur by an alternate pathway of galactose metabolism mediated by the enzyme, uridine diphosphogalactose pyrophosphorylase.

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# Pentose Metabolism and Pentosuria\*

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THE discovery of pentose in material of animal origin occurred in 1892 when Salkowski and Jastrowitz [1] detected the first recognized case of pentosuria. It was two years later that Hammarsten [2] found a pentose in

pancreatic tissue. The present stimulus for research on pentose metabolism derives largely from interest in nucleic acids, the metabolism of which is believed to contain the keys to cell duplication, virus action, and perhaps neoplastic disease as well. The differences in distribution and function of the two types of nucleic acids, the ribonucleic acids and the deoxyribonucleic acids, led, among other things, to intensive studies of the biosynthesis of their respective pentose sugar components. These investigations have had ramifications in many areas of metabolism. Although direct application of the new knowledge to clinical problems has not yet been extensive, it has naturally had a bearing on the study of pentosuria.

The present understanding of pentose metabolism will be discussed briefly before consideration of the pentosurias.

## THE PENTOSE PHOSPHATE PATHWAY

Until a few years ago the Embden-Meyerhof glycolytic pathway and the Krebs tricarboxylic acid cycle were virtually the only bases for the consideration of major problems in the intermediary metabolism of carbohydrates. The glycolytic pathway (Fig. 1) was deemed almost completely responsible for the breakdown of glycogen, glucose, and probably other hexoses to lactate and pyruvate, the latter then being oxidized to carbon dioxide and water via the Krebs cycle. Glycolysis effects the conversion of glucose to two molecules of pyruvate with the production of eight molecules of high energy phosphate as adenosine triphosphate (ATP). On the other hand, the oxidation of the two molecules of pyruvate produces thirty ATP molecules. It is apparent, then, that the Krebs cycle is the one primarily responsible for the ultimate production of useful energy.

Space does not permit elaboration of side paths of the glycolytic route which permit the assimilation of dietary carbohydrates such as fructose and galactose, as well as the production

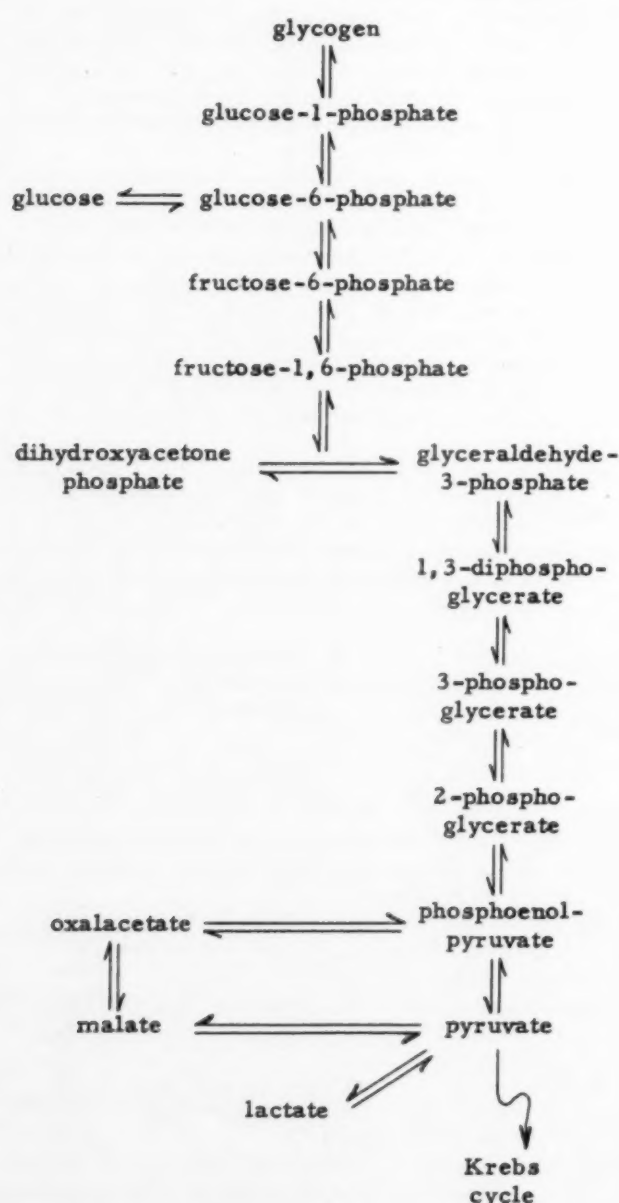


FIG. 1. The Embden-Meyerhof glycolytic pathway.

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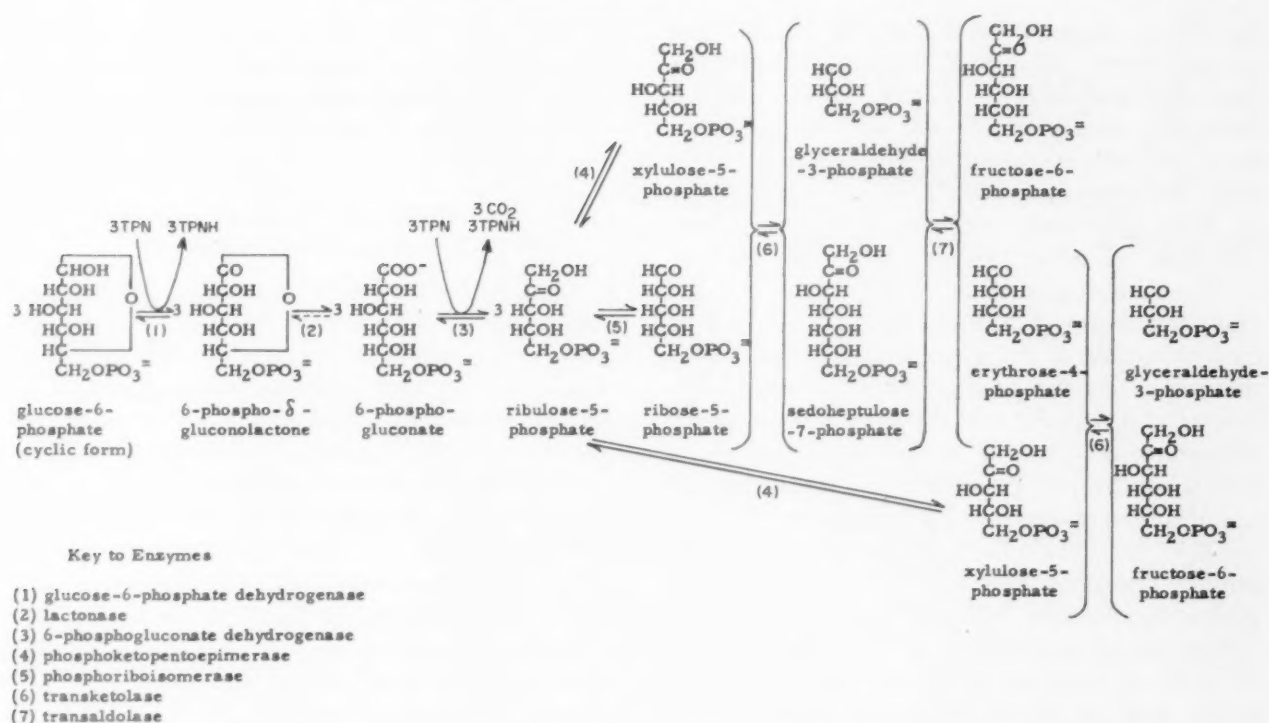


FIG. 2. The pentose phosphate pathway of glucose-6-phosphate oxidation.

of amino sugars. However, the discovery by Warburg, Lipmann and Dickens of an alternative pathway for the metabolism of glucose-6-phosphate, and studies by Dische on ribose phosphate, paved the way for a major advance in our understanding of pentose metabolism in mammals. The observations of these investigators between 1936 and 1938 were followed up after World War II by Cohen, Horecker, Racker, and others [3,4]. A new pathway for carbohydrate oxidation was evolved which, although still being evaluated in regard to its importance in the production of energy, is the main basis for our understanding of pentose formation and utilization. This cycle is most commonly known as the pentose phosphate pathway, but it has also been referred to as the 6-phosphogluconate oxidation pathway and the hexose monophosphate shunt. It is shown in Figure 2.

Steps 1 and 3 are oxidative and are linked to the coenzyme triphosphopyridine nucleotide (TPN); the implications of this coenzyme requirement will be discussed subsequently. Unlike the glycolytic pathway, the pentose phosphate pathway involves the conversion of a sugar carbon atom to carbon dioxide (step 3). Subsequent steps are isomerizations and group transfers involving pentoses and even four carbon and seven carbon sugar phosphates. Elabo-

ration of these hitherto unsuspected transformations was a major achievement indeed. It should be pointed out that the enzyme transketolase, which catalyzes two steps, has thiamine pyrophosphate as coenzyme, thus providing new insight into the physiological role of the vitamin. Three pentose phosphates are intermediates, namely, D-ribulose-5-phosphate, D-ribose-5-phosphate, and D-xylulose-5-phosphate. The relevance of this to the mechanism of formation of the pentose components of nucleic acids and to the metabolism of the pentose excreted in essential pentosuria is discussed later in this review.

It may be noted that, in the pentose phosphate pathway, C-1 of glucose is converted to  $\text{CO}_2$  whereas other carbon atoms are returned to the glycolytic pathway as fructose-6-phosphate and as glyceraldehyde-3-phosphate. On the other hand, when hexose is metabolized exclusively by the glycolytic route, it is split symmetrically to form eventually two identical pyruvate molecules which are oxidized in the tricarboxylic acid cycle. As proposed originally by Bloom and Stetten [5], the difference in the relative rate of liberation of C-1 and of C-6 as  $\text{CO}_2$  has been the basis of most tests to determine the contribution of each pathway to the net oxidation of carbohydrate in mammals. Different experimental

techniques have yielded varying results; the complications in these studies have been discussed elsewhere [6-8]. In an attempt to overcome certain objections, such as that liver slices do not represent a physiological situation, Murphy and Muntz [9] have employed a liver perfusion technique which allows injection of either glucose-1-C<sup>14</sup> or glucose-6-C<sup>14</sup>, attainment of a steady state in each case, and removal of products. The results indicate that half of the glucose is metabolized in this organ via the pentose phosphate pathway. Actually, it is likely that the extent of glucose-6-phosphate and gluconic acid-6-phosphate oxidation is, under physiological conditions, dependent upon the coenzyme concentrations and upon the need for certain biosynthetic operations. The pentose phosphate pathway, unlike the glycolytic pathway, requires TPN for hexose catabolism. Many synthetic processes require reduced TPN (TPNH). Fatty acid synthesis involves a TPNH-linked step, although fatty acid oxidation uses DPN rather than TPN. Even glycogen synthesis from pyruvate in the liver is now considered to require TPNH in the preliminary conversion of pyruvate to phosphoenolpyruvate via malate and oxaloacetate [10,11]. Hence, the extent of glucose oxidation via the hexose monophosphate shunt is likely to be extremely sensitive to physiological needs and experimental conditions. Rat liver homogenates synthesize more fatty acids and cholesterol from acetate if a TPNH generating system is present [12]. Conversely, with rabbit liver slices, it has been shown that C<sup>14</sup>O<sub>2</sub> production from glucose-1-C<sup>14</sup>, as compared with glucose-6-C<sup>14</sup>, is increased in the presence of a TPNH-oxidizing system [13].

Interesting studies have been made in tissues other than the liver. Very recently, almost a complete dependence on a C-1 oxidation pathway has been found in bovine corneal epithelium, and the rate of glucose oxidation was increased markedly by the addition of a TPNH acceptor [14]. In mammary tissue, too, there is now abundant evidence that the pentose phosphate pathway is of major importance in the net oxidation of carbohydrate [15].

Studies in the intact animal [16] indicate that almost all glucose is catabolized via the glycolytic pathway. It may be concluded that the pentose phosphate pathway is normally limited by meager cellular mechanisms for disposing of TPNH and that its quantitative contribution for glucose disposal increases when TPNH utilizing

systems are most active. Obviously, such an adjustment will be limited to those tissues, such as the liver, which have systems important for the biosynthesis of metabolites by TPNH-linked enzymes.

#### THE GLUCURONATE-XYLULOSE OR C-6 OXIDATION PATHWAY

During the last few years another pathway of carbohydrate metabolism has been uncovered. Its elucidation was primarily the result of studies on the biosynthesis of L-ascorbic acid in the rat and on the biochemistry of L-xylulose, the sugar excreted in essential pentosuria.

It has long been known that the excretion of L-xylulose by pentosurics is enhanced by the administration of D-glucuronolactone [17]. It was later shown that the latter substance also stimulates xylulose excretion in normal human subjects and guinea pigs [18,19] and that man and animals, even eating normal diets, may excrete traces of this pentose [18-20]. It has also been shown that the biosynthesis of L-ascorbic acid from D-glucose proceeds by way of D-glucuronate and L-gulonate [21-24]. (Although the anions are apparently the true enzymatic substrates, absorption and permeability factors often make the lactones much more effective.)

In addition to these transformations, an enzymatic pathway for the interconversion of L-xylulose and D-xylulose via xylitol was found in liver [25-27]. Together with the report [28] of a D-xylulokinase in calf liver, these investigations led to the hypothesis that a third carbohydrate pathway exists in mammals [29,30]. This is shown in Figure 3. It should be noted that this pathway is linked to both the pentose phosphate and glycolytic pathways through xylulose-5-phosphate and fructose-6-phosphate.

All tracer and enzymatic studies are consistent with the formulation of the cycle as shown. For example, the administration of labeled L-gulonate to the rat [31] and of xylitol-1-C<sup>14</sup> to the rat or guinea pig [29] give the expected labeling patterns in the carbon chains of liver glycogen. Similarly, the administration of D-glucuronolactone-1-C<sup>13</sup> to a person with essential pentosuria yields the predicted labeling of C-5 of urinary L-xylulose [32].

The formation of free D-glucuronate can conceivably occur in one of two ways. There are liver enzymes for the conversion of glucose-1-phosphate into uridine diphosphate glucose and then into uridine diphosphate glucuronic acid

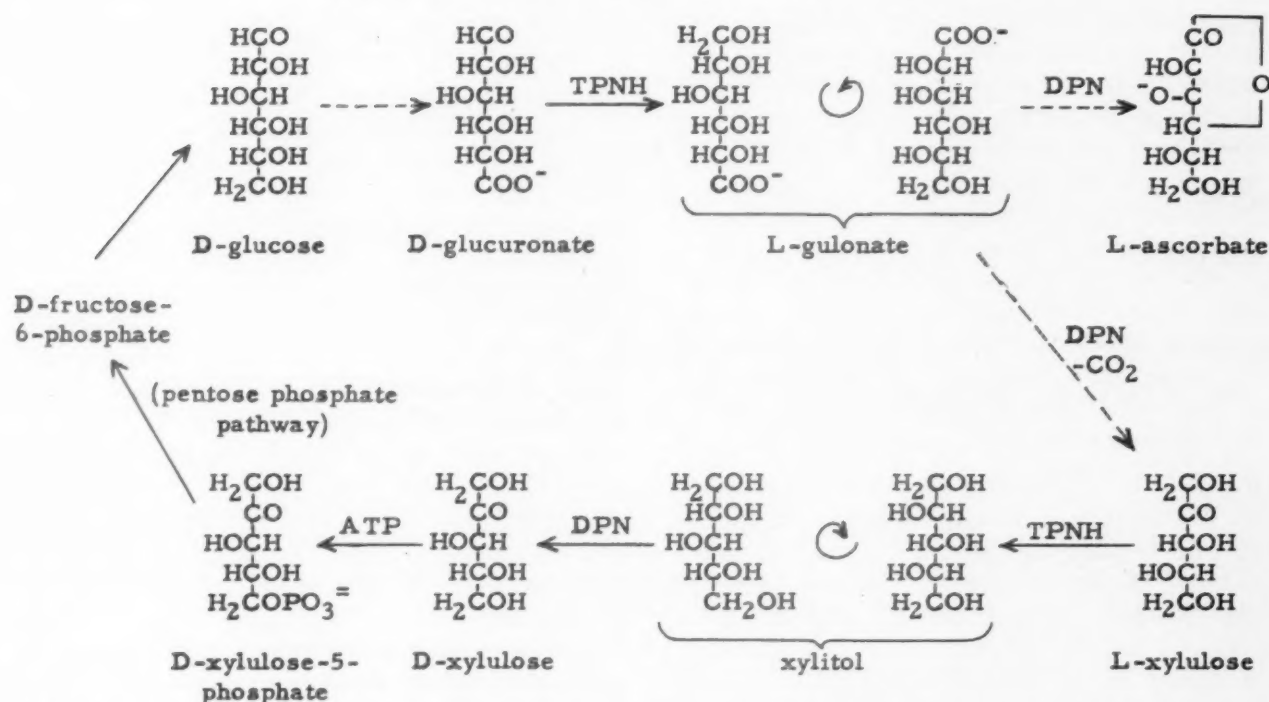


FIG. 3. The glucuronate-xylulose pathway. The broken arrows indicate transformations in which mechanisms are not clearly established.

[33]. Enzymatic hydrolysis of the latter to free glucuronate has been reported briefly [34], but the specificity of the transformation and its relevance to the present questions remain to be determined. If this sequence of reactions is in fact the source of glucuronate, then glucose-1-phosphate rather than glucose would be an intermediate in the cycle. (Fig. 3.) In addition, inositol, a biological product of glucose, is converted into D-glucuronate by an enzyme of rat kidney, thus providing another possible source for the glucuronate which passes through the cycle [35].

The physiological importance of this pathway is not yet clear, except in a species such as the rat which can make its own L-ascorbic acid. It has been shown that the ascorbic acid-forming enzymatic reaction is present in liver microsomes of the rat but not of man, primates, or the guinea pig [24,36]. Tracer studies indicate that little glucose is catabolized via this pathway [37]. It is an intriguing fact that certain drugs, like barbitol and Chloretone®, apparently induce increased metabolism of glucose via the cycle and that hypophysectomy abolishes the drug effects [38]. These results certainly warrant further study.

A possible function of the cycle may involve the coenzymes used in the transformation of

D-glucuronate into L-xylulose and of L-xylulose into D-xylulose. These reactions effect the conversion of TPNH and DPN into TPN and DPNH. In view of the very limited capacity of mitochondria to oxidize TPNH, this might serve as a mechanism for moving the H from TPNH to DPN. It should be emphasized that there are tissue carbohydrates, the origins of which are still unknown and that known pathways may ultimately be found to be more useful in the production of essential metabolites than for the production of energy.

#### THE BIOSYNTHESIS OF D-RIBOSE AND D-2-DEOXYRIBOSE

D-Ribose and D-2-deoxyribose (Fig. 4) are the principal sugar constituents of ribonucleic acids and deoxyribonucleic acids, respectively. Considerable attention has been given to the mechanism by which they are formed.

In the pentose phosphate pathway, D-ribose-5-phosphate is formed from D-ribulose-5-phosphate, which is produced in three steps from glucose-6-phosphate. (Fig. 2.) D-Ribose-5-phosphate can also be considered as the product of transaldolase and transketolase reactions originating at D-fructose-6-phosphate and D-glyceraldehyde-3-phosphate. These enzymes are present in muscle as well as in liver, although the



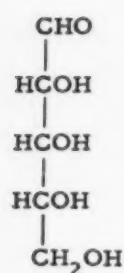
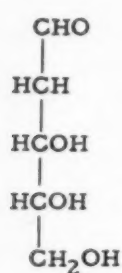
D-riboseD-2-deoxyribose

FIG. 4. The pentose constituents of nucleic acids.

former lacks the oxidative steps of this pathway [39,40]. The two routes to ribose phosphate may be termed the oxidative and the non-oxidative routes, respectively. Studies to determine whether or not nucleic acid ribose is produced by the pentose phosphate pathway are based on an examination of the labeling pattern in the carbon chain of ribose after administration of glucose and other substances labeled in specific positions. In brief, the results indicate that in man and the rat, ribose is produced by this pathway, the contribution of the non-oxidative route in the rat being larger than that of the oxidative route [41-45].

There appear to be at least two modes of biosynthesis of D-2-deoxyribose in nature. One involves an as yet undetermined mechanism by which D-ribose, probably while in combination with other nucleotide components, is transformed into its deoxy analog. Evidence for this conversion of ribose into deoxyribose without cleavage of the carbon chain has been found in *Neurospora* [46,47], in *Escherichia coli* [48-50], and in the rat [51-54]. Moreover, the direct conversion of ribonucleosides or nucleotides into corresponding deoxy analogs has been reported in cell-free bacterial extracts [55,56], chick em-

bryo preparations [47], and Ehrlich ascites cells *in vitro* [58].

There is also evidence for an entirely different mechanism. In 1952 Racker [59] reported that an enzyme (deoxyribose phosphate aldolase) from *Esch. coli* catalyzes the formation of D-2-deoxyribose-5-phosphate through an aldol condensation of acetaldehyde and D-glyceraldehyde-3-phosphate. (Fig. 5.) Presumptive evidence for this route has been reported for *Bacillus cereus* [60]. The enzyme has been found in guinea pig liver [61], in mouse liver and thymus [59], in normal rat liver [62] and in induced and transplanted hepatomas of the rat [62]. Moreover, following the administration of labeled glycine, the deoxyribose in rat liver deoxyribonucleic acid is reported to have a labeling pattern conforming to the Racker mechanism [63].

The question of deoxyribonucleic acid synthesis in malignant tissue is being approached by Boxer and Shonk [62] by studying deoxyribose-5-phosphate metabolism. All tumors studied have increased capacity to form but decreased ability to utilize the substance, while in regenerating liver there is an increase in capacity for both synthesis and utilization. The authors are cautious about drawing inferences from these results because of the complex nature of their enzyme preparations. However, they have already been able to separate the deoxyribose phosphate aldolase from the system responsible for disappearance of the deoxypentose phosphate. Further studies of this type should prove extremely valuable, whether or not these reactions are actually important in the formation of deoxyribonucleotides.

Using specifically labeled glucose or ribose, Horecker et al. [64] found fairly similar labeling patterns in the ribose and deoxyribose of the nucleic acids of regenerating liver, ascites tumor

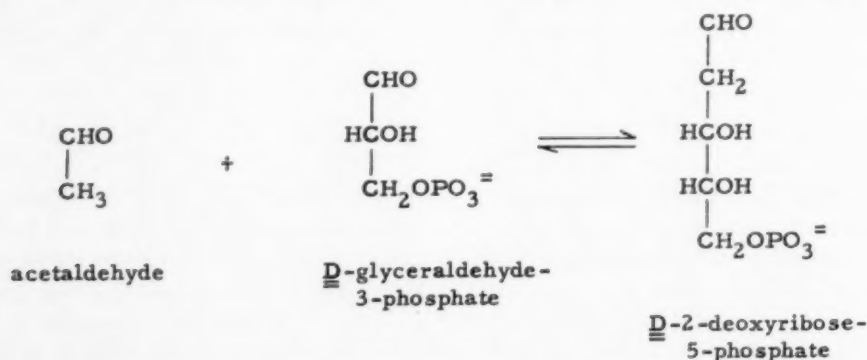


FIG. 5. Reaction catalyzed by deoxyribose phosphate aldolase.

cells, and HeLa cells. However, experiments with glucose-1-C<sup>14</sup> yielded much higher labeling of C-5 of deoxyribose as compared with ribose. This result suggested possible involvement of a triose phosphate in deoxyribose formation, but the labeling pattern was considered to rule out its condensation with acetaldehyde.

It is possible that some conflicting results will eventually be explained by differences in experimental procedures. However, it is not unlikely that variability in the biosynthetic route will be found among different species and perhaps even among different tissues of the same animal. Where enzymatic mechanisms for two biosynthetic pathways are found, physiological factors such as coenzyme concentration may determine which is quantitatively the more important.

#### ALIMENTARY PENTOSURIA AND THE UTILIZATION OF PENTOSE

Investigation of pentosuria requires awareness that dietary factors can influence pentose excretion in normal persons with no biochemical anomalies. Since many fruits contain considerable amounts of pentoses metabolized with difficulty, fruit-free diets are often necessary in studies of persons suspected of having pentosuria of physiological or pathological origin.

Direct studies on the utilization of pentoses have been few in number and the results obtained have not always been in agreement with each other. For present purposes it is sufficient to mention that easily detectable pentosuria was shown in 1906 to occur in most but not all subjects from the ingestion of 1.5 L. of apple juice [65]. The variation in susceptibility to alimentary pentosuria may be related to the capacity of the kidneys to reabsorb sugars. It has been shown that xylose and glucose are reabsorbed by the same transport system [66]. Thus, as has been pointed out by Knox [67], the report [68] of the coexistence of renal glycosuria with susceptibility to alimentary pentosuria is not surprising. It has been shown by paper chromatography that fruit-free diets reduce to less than half the normal excretion of aldopentoses, the reduction being primarily in xylose and arabinose, that of ribose being only slightly lower [69]. That there are many unsuspected regulators of pentose excretion is suggested by the finding that low temperatures and thyroid treatment increase urinary pentose excretion in

rats [70,71]. The cold stress is counteracted by ACTH, and thiouracil reduces pentose levels.

Since different pentoses vary in their capacity to be metabolized, they should be considered individually in investigative work. Studies on aldopentoses were reviewed in 1954 [72], and the utilization *in vivo* of very small doses of labeled aldopentoses were studied more recently [29,73]. The limited utilization of a large oral dose of D-xylose, with the resulting excretion of a considerable quantity in the urine, is the basis for its use in studying malabsorption syndromes [74].

The cataracts produced in galactosemia, in experimental diabetes, and by feeding animals diets containing a high concentration of D-xylose or D-galactose have stimulated biochemical studies of the mechanisms involved. The recent work of van Heyningen [75], showing that the administration of D-xylose to the weanling rat causes accumulation of xylitol in the lens but not in other tissues, and that calf lens contains enzymatic systems for the conversion of xylose to xylitol [75] and xylonic acid [76], indicates the existence of interesting facets of carbohydrate metabolism still to be uncovered.

#### THE QUESTION OF PENTOSURIA IN NEUROMUSCULAR DISEASE

In 1949 Minot et al. [77] reported that patients with progressive muscular dystrophy excrete abnormal amounts of ribose which appeared to occur in a labile organic phosphate complex. The ribose reduced Benedict's solution only after forty-five minutes in a boiling water bath, presumably because of its linkage in the complex. Reaction with phenylhydrazine yielded ribosazone from the urine of patients with progressive muscular dystrophy of various types, dystrophia myotonica, myotonia congenita and amyotonia congenita, but not from patients with other neuromuscular disorders [78].

These intriguing findings recalled the 1907 report [79] of an association of pentosuria with neurologic disease; this could not subsequently be confirmed [80]. Support for the clinical findings of ribosuria was obtained by Minot and Grimes [81] when they found, in rabbits, that muscular dysfunction induced by vitamin E deficiency was accompanied by the simultaneous appearance in the urine of pentose and readily hydrolyzable organic phosphate.

Attempts to reproduce the clinical results of Minot have, in general, been discouraging. Only limited support came from studies on

pentosazone isolation from urine [82,83], and paper chromatography failed to show abnormal amounts of free or bound ribose in the urine of dystrophic patients [84,85]. It should be pointed out that the original characterization of the urinary pentosazone [78] was inconclusive in that its failure to depress the melting point of D-ribosazone was not a definitive test. Mixtures of different pentosazones do not always show the melting point depression which is usual when two different substances are mixed [86].

In 1956 three studies were reported which led to different conclusions. One [69] found significantly higher ribose excretion in muscle disease, the urinary ribose being inversely proportional to residual functional muscle mass as determined by potassium exchange. However, the urinary pentose was less than 0.1 gm. per day, far less than the amount found in essential pentosuria, and acid hydrolysis did not liberate any free aldopentose. A second study [87], employing paper chromatographic examination of urine specimens obtained on hospital visits rather than 24-hour collections, found no obvious differences in the distribution or amount of pentose excreted by the normal and dystrophic groups. The third study [88] found only slight differences in osazones derived from normal and dystrophic urines and concluded that any differences in pentose excretion between the two groups seem to be too small for characterization by present methods. All these investigations indicate that the original observations of Minot still require explanation.

#### ESSENTIAL PENTOSURIA

Essential pentosuria may be defined as the inherited disorder characterized by the excretion of amounts of pentose, usually more than 1 gm., which is large compared to the milligram quantities of various sugars excreted daily by normal human subjects. The identification of the urinary pentose has been of utmost importance in the clarification of the confusing state of affairs that existed for many years. The early literature on pentosuria is beclouded by studies employing inadequate chemical procedures and by conflicting hypotheses regarding its physiological or pathological basis. However, it is now possible to discuss the detection, frequency and clinical importance of pentosuria with greater certainty. Furthermore, the valuable biochemical information which has been obtained gives promise that remaining questions about the nature of the defect will soon be answered.

Although the occurrence of pentosuria was clearly recognized in 1892 [7], a case of glycosuria had been detected in 1880 that was later identified as an example of pentosuria [89,90]. As many additional cases came to clinical observation the genetic origin of the disorder became so evident that Garrod included pentosuria in his Croonian Lectures of 1908 on "Inborn Errors of Metabolism" [97].

*The Characterization of the Urinary Sugar.* Differentiation of pentose from the more commonly encountered glucose has presented little difficulty, since the former is not fermentable by yeast, gives a lower melting osazone with phenylhydrazine, and responds to the orcinol test of Bial. In 1900 Neuberg [92] reported that the pentose in essential pentosuria has properties consistent with *dl*-arabinose, that is, the racemic, optically inactive mixture of the two forms of this sugar. For example, he obtained the diphenylosazone of arabinose from pentosuric urine. Several other investigators reported cases of arabinosuria [93-96], often with rather convincing evidence. Cammidge and Howard [94], in 1920, oxidized the urinary pentose to *dl*-arabonolactone and resolved the latter into its component optically active forms. It had frequently been mentioned, however, that pentosuric urine is dextrorotatory [97], a property which led some to believe that the urinary sugar was *l*-arabinose [98]. Inability to obtain a derivative with diphenylhydrazine [99,100] cast further suspicion on the previous generalizations about the identity of the urinary sugar.

In 1913 Zerner and Waltuch [101,102] obtained convincing evidence that one type of essential pentosuria involves the excretion of a substance related to xylose rather than arabinose. They exploited the fact that, whereas D- and L-xylose phenylosazone (xylosazone) melt at about 161°C., DL-xylosazone melts near 205°C. These investigators were unable to obtain positive tests for arabinose in pentosuric urine, but succeeded in isolating L-xylosazone, as shown by its melting point alone and in mixture with the D isomer. It should be mentioned here that (1) because *d* and *l* have at times been used to indicate direction of rotation of polarized light rather than absolute configuration, D and L are now used when configuration is known, and (2) Emil Fischer's classification of sugars gave the reversed notations for xylose. Hence, Zerner and Waltuch really reported that the osazone of the urinary pentose is related to *d*-xylose,



which is now known as L-xylose. Levene and LaForge [86] quickly confirmed this work and, in addition, showed that the urinary pentose is a ketose. Their designation, *d*-xyloketose, has gradually been replaced by the more proper one, L-xylulose. (The formula is shown in Figure 3.) There is now abundant confirmation of these chemical studies [103–106].

Although the question of arabinosuria has not been completely resolved, it no longer receives much attention. Two cases reported in 1910 [107] and in 1913 [93] were later shown to involve xylulose excretion [97,108]. Moreover, new cases of xylulosuria have been encountered quite frequently in the last few decades, but there have been none of arabinosuria [97]. The absence of optical activity of some urine specimens containing L-xylulose may be due to the compensatory activity of a levorotatory constituent [106]. In spite of the overwhelming preponderance of xylulosuria among pentosurics, however, it is still sometimes (erroneously) stated that the urinary sugar is usually arabinose [109].

*The Detection of Essential Pentosuria.* The detection of essential pentosuria is simple. There is no excuse for treating patients with pentosuria as diabetic patients, although examples of such misdiagnosis have been reported in the fairly recent literature [110,111]. The non-fermentability of urine which reduces Benedict's solution, or the association of a normal glucose tolerance curve with glycosuria, especially in a Jewish patient, should lead to a consideration of pentosuria. Making use of earlier observations, Lasker and Enklewitz [112] in 1933 described a semi-quantitative test for urinary xylulose based on its rapid reduction of Benedict's solution at 55°C. Under the conditions employed, aldose sugars do not respond, and other ketoses, such as fructose, reduced the reagent less rapidly. However, since fructosuria is a very rare anomaly, the reaction of fructose is of minor significance. Its occurrence could be determined by the Seliwanoff test or by paper chromatography.

Confirmation of the presence of considerable quantities of xylulose in the suspected urine can be made by paper chromatography [111,113], and by the osazone melting point test mentioned in the previous section. The mixed osazone test is necessary because the pure osazones of all pentoses have practically the same melting point. These procedures provide a combination of simplicity and reliability not always characteristic of color tests in vogue earlier. However, it should

be noted that arabinose does not respond to the low temperature Benedict's test and would require other methods, such as paper chromatography, for its identification. The question of arabinosuria and its detection was reviewed by Lasker in 1950 [97].

The amount of xylulose excreted by pentosurics on normal diets is rather constant for each person, usually being between 2 and 4 gm. per day and varying from day to day by less than 0.3 gm. [19,114]. Early reports of larger amounts were most likely based on results with patients under medication or on questionable chemical methods. The amount of pentose excreted is lowered by about 50 per cent by fasting [106,115] but seems to be unaffected by ordinary dietary constituents. The daily output appears to be dependent primarily upon body weight [106].

*Precursor of the L-Xylulose.* As cases of essential pentosuria were encountered by various workers, attempts were made to determine a dietary or metabolic influence on pentose excretion. Since conflicts among conclusions drawn from these studies may in part have been a result of the confusion between xylulosuria and arabinosuria, the earlier work will not be discussed in detail. Briefly, the prevalent opinion in the nineteen twenties was that protein is the most likely source of the urinary pentose [116–118], but the evidence for this was quite meager.

Clarification of this question evolved from clinical observations and a rather interesting hypothesis. The first reported case of pentosuria occurred in a morphine addict [7], and many subsequent reports suggested a relationship between this drug and pentose excretion [118]. In 1929 Margolis [118] showed that other drugs, such as aminopyrine, also stimulate pentose excretion by pentosurics. Enklewitz and Lasker [106] confirmed this and offered the fruitful hypothesis that there may be a relationship between L-xylulose excretion and the stimulation of glucuronic acid formation by drugs which are "detoxified" by conjugation with this substance. Their work was climaxed by the demonstration that D-glucuronolactone itself leads to a marked enhancement of xylulose excretion by pentosurics [17], although a normal subject was not similarly affected. It should be pointed out that glucuronic acid is almost always used as the readily available lactone rather than as the free acid or as a salt. An experiment on the enhancing effect of glucuronolactone is shown in Figure 6.

In the light of present knowledge, it seems

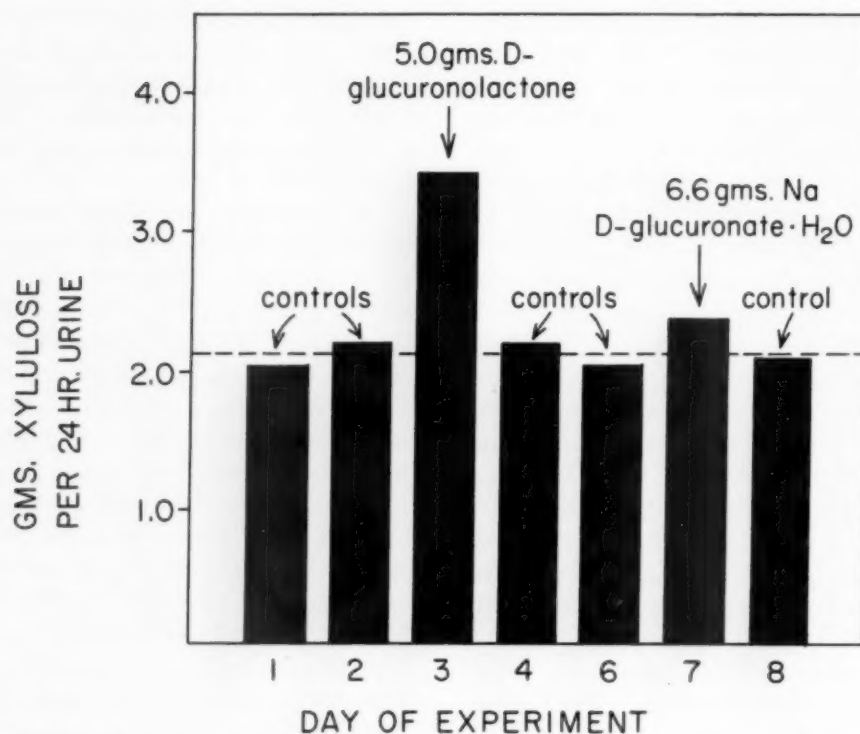


FIG. 6. Effect of orally administered D-glucuronolactone on L-xylulose excretion in the pentosuric subject of Touster et al. [19]. Note the constancy of daily xylulose output. The negligible effect of sodium glucuronate, as compared to the lactone, is undoubtedly a result of poor absorption or low tissue permeability.

likely that this valuable finding does not really confirm the Enklewitz and Lasker hypothesis since, as has been pointed out elsewhere [19], aminopyrine has a much greater effect on xylulose excretion than can be accounted for by the small amount of the drug which is detoxified by glucuronide formation. D-Glucuronolactone, on the other hand, increases urinary pentose levels to an extent consistent with its actually serving as a precursor.

Until fairly recently, biochemical knowledge was inadequate to provide an explanation for such a transformation. The application of sensitive chromatographic procedures made it possible for us to show that glucuronolactone enhances xylulose excretion in normal humans and guinea pigs [18,119]. Use of labeled glucuronolactone demonstrated that, in the pentosuric human, the conversion to L-xylulose is a rather direct one and involves loss of the carboxyl carbon [32]. It now seems likely that urinary xylulose in both pentosuric and normal persons derives from hexose, as shown in Figure 3. It is necessary to point out, however, that this has not been tested directly by showing that labeled glucose, as well as administered glucu-

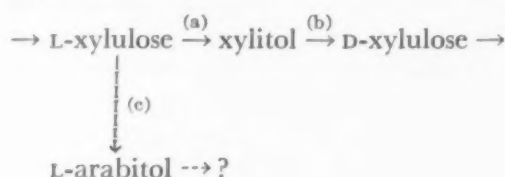
ronolactone, labels the xylulose precisely as predicted by the postulated cycle.

*Nature of the Defect.* A considerable number of explanations have been offered for the biochemical abnormalities of genetic origin. They have been reviewed very recently [119,120], and need not be fully discussed here. It may be mentioned, however, that whereas galactosemia has clearly been shown to involve a deficiency of a normal enzyme component of a metabolic pathway [121], cystinuria [122] and renal glycosuria are defects in renal absorption mechanisms.

In considering the underlying defect in essential pentosuria, it is well to emphasize that there is no evidence that, as has already been suggested [123], L-xylulose is an abnormal metabolic product. The isolation of xylulose from normal human and animal urine has already been mentioned, as well as the fact that animal liver contains enzymes which can catalyze its formation and utilization. There has long been abundant evidence that the pentose is utilized *in vivo*. The finding of little or no xylulose in urine led to the conclusion that it is metabolized by normal human subjects after oral administration [106] and by the mouse [18], rabbit [106] and

dog [124] after parenteral administration. In depancreatized dogs it was shown that orally and intraperitoneally administered L-xylulose led to increased urinary glucose [125]. Surprisingly, a patient with pentosuria fed 5 gm. of L-xylulose excreted only 0.5 gm. more than usual [106].

It has usually been assumed that, in essential pentosuria, there is an enzyme deficiency in a metabolic pathway. If this is true, it would seem likely that the lack of TPN-xylitol(L-xylulose) dehydrogenase (step a) prevents the conversion of L-xylulose to xylitol:



However, until very recently, a block between xylitol and D-xylulose (step b) was possible. In this case, both xylitol and L-xylulose would probably be excreted, but the xylitol would have remained undetected by the procedures commonly employed because, lacking a carbonyl group, it does not respond to tests specific for sugars. It therefore appeared desirable to examine pentosuric urine for the presence of this pentitol. Extensive chromatographic purification of a periodate-positive fraction of the urine disclosed the presence of a pentitol, but it proved to be L-arabitol rather than xylitol [126]. This finding, while eliminating the possibility of a block between xylitol and D-xylulose, raises other questions. The urinary L-arabitol, which was found in amounts approximating a daily output of a few hundred milligrams per day, may simply be the result of the direct reduction of accumulated L-xylulose by an unimportant enzyme or by one whose normal function is to catalyze some other reaction. On the other hand, the excretion of L-arabitol, which also occurs to a smaller extent in non-pentosuric persons [126], may reflect the presence of hitherto unsuspected metabolic reactions. A direct attack on these problems would include estimation of the L-xylulose-xylitol enzyme in the liver of normal persons and persons with pentosuria.

A renal mechanism for essential pentosuria remains open for consideration. It has received very little attention in the past, but a recent review [67] has wisely emphasized the need for proper examination of this possibility. A "meta-

bolic defect" should be associated with increased body concentrations of L-xylulose. This probably would be reflected by higher blood xylulose levels, especially after the administration of glucuronolactone, in pentosuric persons as compared with normal subjects, unless the renal threshold for it in all human subjects is low or negligible. With a renal defect, on the other hand, the blood xylulose level of the pentosuric subject may be lower, and after administration of glucuronolactone it should not rise as much as in the normal subject. Flynn [177], using paper chromatography, has shown that pentosuric serum contains a small amount of xylulose which is augmented by glucuronolactone administration. Unfortunately, a similar study of normal persons was not made.\*

The conversion of labeled D-glucuronolactone to D-ribose in a non-pentosuric human subject has been shown to occur in a manner consistent with the pathway in Figure 3, but the transformation did not take place at all in a patient with pentosuria [127]. In view of the well established conversion of D-glucuronolactone to L-xylulose in pentosuria, a "block" in the utilization of this pentose again seems likely. Whether this results from a metabolic defect or from a renal defect remains to be determined. Moreover, there is still some contradiction between the excretion, in fairly good yield, of L-xylulose formed by patients with pentosuria from administered glucuronolactone [17,19,32], and the finding that xylulose given orally to a patient with pentosuria led to only a small increase in urinary pentose [106]. However, the latter result was obtained from an experiment in only one subject. There may be quantitative or even qualitative differences in the basic defect among persons with this anomaly.

*Genetic Origin of the Disorder.* As reports of pentosuria were added to the literature, it became evident that essential pentosuria occurred frequently in the same family. Thus, in 1929 it was stated that "24 or about one-third of all the cases reported to date occurred in nine families" [118]. It was usual for the case histories to state that the patients were Jews. In 1933 Enklewitz

\* Preliminary studies performed on pentosuric subject I. B. show marked elevation of blood xylulose levels after ingestion of glucuronolactone. Since the blood xylulose did not respond similarly in a normal subject, it appears that the pentosuria defect is not a renal one. Confirmatory and quantitative experiments are in progress. (BOZIAN, R. C. and TOUSTER, O. Unpublished work.)



and Lasker [106], employing reliable chemical procedures, identified twelve persons as L-xylulosurics; all were Jews. At the present time, there are some 200 recorded cases of essential pentosuria. Omitting a few of the reported instances of arabinosuria, all except two patients for whom appropriate information is given are of Jewish origin. The two are said to be non-Jewish Lebanese sisters living in South Africa [113]. Two of the seven patients with arabinosuria reported on in England in 1920 were in the same Greek family, two others were "purely English by birth and ancestry," and the other three were Jews [94]. One other non-Jewish patient with arabinosuria has been mentioned [128].

The incidence of essential pentosuria is difficult to estimate precisely because, as a harmless entity, many cases probably have not been reported or have not even come to medical attention. Pentosuria has also been diagnosed as renal glycosuria, although the differential diagnosis is simple. Furthermore, results from urban population centers with a relatively high percentage of persons of Jewish origin should indicate a greater frequency than in other areas. The evaluations of Greenwald [116] and of Margolis [118] suggest an incidence of perhaps one in a thousand, but the summary of Blatherwick (quoted in [129]) led him to suggest a lower frequency. Among 130,985 specimens of urine obtained from life insurance applicants and employees of the insurance company involved, 8,318 specimens with a sugar content of 0.25 per cent or more were tested for the presence of xylulose by the modified Benedict's test of Lasker and Enklewitz. The thirty-one cases of xylulosuria found give an incidence of one in 4,225. Since eleven urine analyses were made in the field for each one in the laboratory, the incidence of pentosuria was concluded to be about one in 50,000 [130]. It seems meaningless to the present author to give such a figure for the general population, since the percentage of Jewish persons is of such crucial significance. Therefore, if only the laboratory results mentioned are considered, and if it is borne in mind that pentosuric urine not infrequently contains less than 0.25 per cent of xylulose, an incidence of much greater than one in 4,000 seems probable among Jews. The earlier estimates of one in a thousand may be much more valid.

The only thorough examination of the genetics of this anomaly was made by Lasker et al. [129]. Their study of thirty-seven personally observed

cases (all excreted L-xylulose) in twenty families provided evidence of recessive heredity. In an analysis of the incidence of the anomaly among siblings of pentosuric persons of ten families in which neither parent was affected, the result conformed with that expected from a recessive Mendelian characteristic. Recessiveness was also indicated by the frequency of consanguineous marriages in families in which there were pentosuric persons. A considerable majority of the recorded cases are male, yet this preponderance may only reflect the greater frequency with which males appear for life insurance examinations [118]. This explanation is supported by the fact that when brothers and sisters of pentosuric subjects were tested, more females were found to excrete xylulose [129]. The evidence was considered to favor the hypothesis that a single autosomal gene is involved. It has been estimated that the probable incidence of the gene for pentosuria in the Jewish population is between one in forty and one in sixty [131]. The view of Schultsz [132] that the condition is transmitted as a dominant trait has not received support.

As already indicated, essential arabinosuria, if it does in fact occur, has no racial predilection. It would be interesting to know whether or not L-xylulose was the urinary sugar in pentosuria found in four of five siblings and their grandmother [133]. The urine of one parent was questionable; the urine of the other parent was negative.

The genetics of pentosuria would be clarified further if heterozygotes could be detected. There is now abundant evidence that two dominant alleles often exert a more pronounced effect than a single one and that there are different degrees of recessivity [134]. Biochemical tests can detect heterozygotes of the recessive anomaly oligophrenia phenylpyruvica [135], and a type of cystinuria designated as an "incompletely recessive" one has been differentiated from another phenotype conforming to the segregation expected in a recessive trait [122]. It is interesting that in a careful analysis of the urine of four "non-pentosuric" persons, two of them Jewish, xylulose amounting to an output of 60 mg. daily was found in one Jew although none could be found in the other subjects [19].

Essential pentosuria has been found in human subjects at all ages, except for very young infants, who would be unlikely to be tested for glycosuria. The earliest reported cases of pentosuria

in young children were actually examples of a rather complicated alimentary pentosuria [136] or of arabinosuria later said to be xylulosuria [93,97]. The latter subject was five years old when pentosuria was discovered. Fischer and Reiner [137] had four pentosuric patients between two and five years old, but the nature of the pentose was not determined. In the two year old glycosuria was noted at twenty-two months. The pentose was also not determined in the urine of a twenty month old patient [138] or of an eleven year old with glycosuria since before the age of two [139]. A recent summary shows that xylulosuria has been found in children as young as two years of age [140].

*Clinical Implications and Drug Effects.* There now is ample evidence that essential pentosuria is not necessarily accompanied by any symptoms of clinical significance. Since several of the earliest cases of pentosuria occurred in morphine addicts, the idea naturally arose that drugs might induce this condition. It is important to note, however, that many drugs are excreted as labile glucuronides which could have been responsible for positive tests for reducing sugars, or even for pentoses when the Bial test was the main diagnostic tool. Moreover, as already pointed out in this review, certain drugs enhance the excretion of xylulose by pentosurics. There have probably been cases of pentosuria with minimal urinary pentose levels in which it has seemed as if the drugs induced the pentosuria [118].

Since pentosuria naturally has been detected very often in persons under clinical examination, there have been a variety of symptoms with which to associate this metabolic error. Margolis [118], after a rather comprehensive survey of the literature in 1929, came to the conclusion that "chronic pentosuria should be classified as a distinct clinical entity, rather than as a harmless anomaly, as is currently believed." He reported an association of pentosuria with migraine and with nervous symptoms of various sorts. However, as Garrod [117] had previously pointed out, the possibility of a diagnosis of diabetes may contribute to morbid symptoms in patients. (This would of course have been much more likely in Garrod's era than in our own.) Some early observers actually suggested that there is a relationship between pentosuria and diabetes mellitus, but the evidence for this was not convincing [106,117,118]. The frequency with which both disorders are found among Jews may have

contributed to this notion. The mortality of seventy-two cases of L-xylulosuria followed for an average of 14.5 years each was found not to differ significantly from that predicted from life tables [140].

There seems to be no present basis for referring to this condition as a disease. It has no known relationship to the health of the person in whom it occurs. The direct importance of essential pentosuria to clinical medicine lies in its differentiation from other glycosurias. Its study contributes to an understanding of human metabolism and genetics, and serves as an analogy for the investigation of the harmful inborn errors of metabolism now broadly referred to as hereditary molecular diseases [141].

#### SUMMARY

The metabolism of pentoses is reviewed. The pentose phosphate pathway (6-phosphogluconate oxidation pathway) is the source of tissue D-ribose and perhaps D-2-deoxyribose as well. In certain tissues it also contributes to the net oxidation of glucose and probably supplies reduced triphosphopyridine nucleotide for biosynthetic reactions. Little is known about the importance of the glucuronate-xylulose cycle, except that from it the L-ascorbic acid produced by the rat is derived. It is significant that L-xylulose, the characteristic urinary sugar of essential pentosuria, is an intermediate in this pathway.

It can be stated with considerable certainty that essential pentosuria is a harmless biochemical anomaly linked to a rare recessive gene which is virtually restricted to individuals of Jewish origin. The exact nature of the defect has not been clearly determined.

The questionable existence of other types of pentosuria is discussed.

*Acknowledgment:* I am indebted to the National Science Foundation, the Muscular Dystrophy Associations of America, Inc., and to the Eli Lilly and Company for support of studies in my laboratory, and to the Howard Hughes Medical Institute for my present Investigatorship. Stimulating discussions with co-workers and colleagues, and the continued interest of Mrs. Margaret Lasker, are very gratefully acknowledged.

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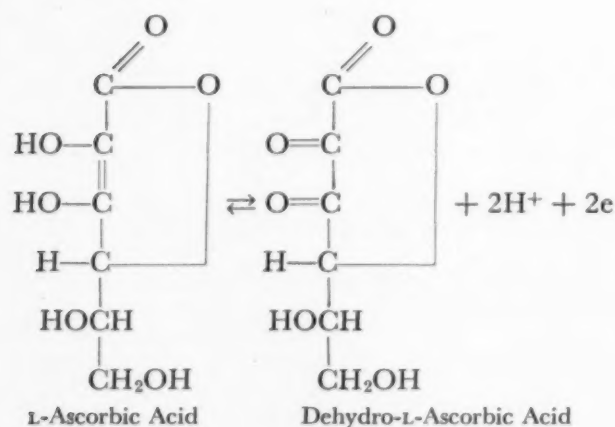
# Biosynthesis of L-Ascorbic Acid; Basic Defect in Scurvy\*

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FOR centuries it has been known that scurvy occurs in man receiving a diet deficient in fresh fruits or vegetables. About twenty-five years ago vitamin C, the antiscorbutic substance, was isolated and identified as L-ascorbic acid. Since that time vitamin C has been investigated extensively, but knowledge of its physiological and chemical functions is far from complete.

L-Ascorbic acid readily undergoes reversible oxidation and reduction to dehydro-L-ascorbic acid as follows:



This property of the vitamin has been of paramount importance in the search for its physiological role. For instance, various biological systems have been described in which L-ascorbic acid is coupled with other redox agents such as glutathione, cytochrome c, pyridine nucleotides and flavin nucleotides. Considerable evidence has been presented that the vitamin can serve in plants as a respiratory catalyst in cellular oxidation and reduction [1], but indications for such a role in animal tissues are meager. Probably the most clearly established functional role of L-ascorbic acid is in maintaining the normal intercellular material of cartilage, dentin and

bone [2] but the biochemical mechanisms involved are still unknown. L-Ascorbic acid has been reported to be involved in the metabolism of various substances including tyrosine [3-5], adrenal steroids [6] and drugs [7]. However, in these systems the vitamin apparently does not act as a conventional co-factor since its requirement can usually be replaced by other compounds with similar redox properties. Although vitamin C is thought to have a beneficial effect in many clinical conditions, the only definitely proved indication for its use is in the cure and prevention of scurvy.

L-Ascorbic acid is synthesized in a wide variety of plants and in all mammals studied except man, other primates and the guinea pig. In recent years considerable information has appeared on the metabolic pathways involved in the formation and degradation of the vitamin.

The present study is concerned primarily with the reactions involved in the biosynthesis of L-ascorbic acid from glucose. The inability of man, monkey and guinea pig to synthesize the vitamin can be traced to the lack of an enzyme system required for the formation of L-ascorbic acid, and it is this metabolic defect which necessitates inclusion of vitamin C in the diet for the prevention of scurvy. Studies of the biosynthesis of L-ascorbic acid have played an important role in revealing a new route of glucose metabolism in animals, the glucuronic acid pathway. The reactions involved in this pathway and their relationship to various disorders of carbohydrate metabolism are reviewed.

## BIOSYNTHESIS OF L-ASCORBIC ACID IN ANIMALS

Studies carried out during the past ten years have disclosed a pathway for the biosynthesis of L-ascorbic acid in rats. (Fig. 1.) Although the

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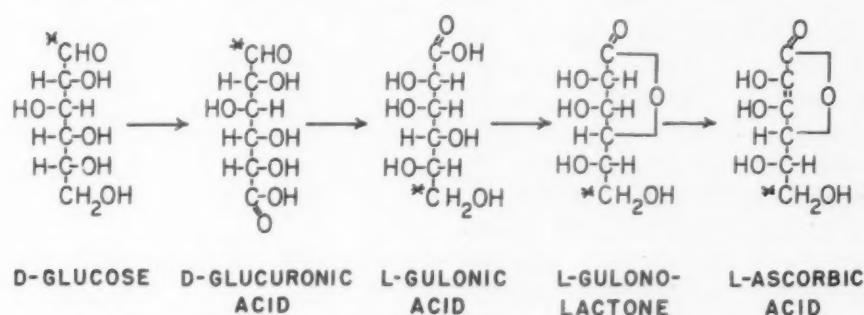


FIG. 1. Pathway for the biosynthesis of L-ascorbic acid in the rat. The asterisks denote the fate of C-1 of D-glucose in this scheme.

possibility that glucose or a related hexose may serve as a precursor of L-ascorbic acid had been suggested from numerous studies, the first direct evidence for such a precursor relationship came from the isotopic studies of King and his associates [8-10]. In these experiments, both C-1 and C-6 labeled D-glucose were administered to rats and the incorporation of C<sup>14</sup> into urinary L-ascorbic acid was determined. D-Glucose-1-C<sup>14</sup> was converted to L-ascorbic acid labeled chiefly in C-6, and D-glucose-6-C<sup>14</sup> was converted to L-ascorbic acid labeled chiefly in C-1. These results indicate that D-glucose is converted to L-ascorbic acid by a mechanism through which its carbon chain undergoes an inversion of configuration.

The pathway in Figure 1 is in accord with the data obtained in the experiments with labeled D-glucose since C-1 of D-glucose would become C-6 of L-ascorbic acid, as indicated by the asterisks. It should be noted that an inversion of configuration of the carbon chain of D-glucose occurs in the reduction of D-glucuronic acid to L-gulonic acid.

Further evidence for the sequence of reactions shown in Figure 1 has come from a number of studies. Isherwood and co-workers [17] reported that administration of D-glucuronolactone and L-gulonolactone to rats produced an increase in the urinary excretion of L-ascorbic acid. Definitive evidence for the conversion of D-glucuronic acid and L-gulonic acid to L-ascorbic acid in rats came from isotopic experiments [12,13]. In addition, the conversion of radioactive D-glucose to urinary D-glucuronic acid and L-gulonic acid has been demonstrated in rats receiving barbital and Chloretone,<sup>®</sup> drugs which are known to stimulate the synthesis of L-ascorbic acid [14-16].

D-Galactose also is converted to L-ascorbic acid in rats [17]. In fact, D-galactose-1-C<sup>14</sup> is a

better precursor of L-ascorbic acid than is D-glucose-1-C<sup>14</sup>. The L-ascorbic acid formed from D-galactose-1-C<sup>14</sup> had about 92 per cent of its total C<sup>14</sup> in C-6 compared to 60 per cent for that from D-glucose-1-C<sup>14</sup>, indicating that D-galactose is converted to L-ascorbic acid without the intermediary formation of D-glucose.

Based upon currently available data, the most likely over-all scheme for the formation of L-ascorbic acid in rats is given in Figure 2. Enzymes are present in rat liver which convert D-glucose or D-galactose to uridine-diphosphoglucose (UDPG) [18-20]. This nucleotide derivative is oxidized to uridinediphosphoglucuronic

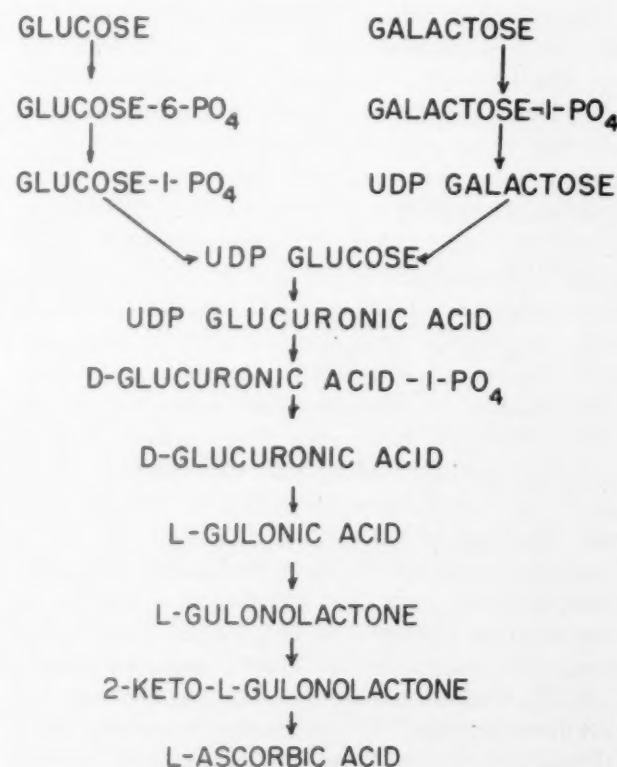


FIG. 2. Over-all scheme for the conversion of D-glucose and D-galactose to L-ascorbic acid in the rat.

acid (UDPGA) by a DPN-linked\* enzyme in the soluble fraction of liver. Another enzyme in liver microsomes utilizes UDPGA to form glucuronides. Recently, an enzyme system has been demonstrated in the particulate fraction of rat kidney capable of converting UDPGA to D-glucuronic acid, presumably via D-glucuronic acid-1-PO<sub>4</sub> [27]. Furthermore, rat liver has the enzymes capable of carrying out the over-all synthesis of labeled D-glucuronic acid from D-galactose-1-C<sup>14</sup> [17]. Of particular importance, an enzyme system is present in liver microsomes which converts UDPGA to D-glucuronic acid-1-PO<sub>4</sub> [17].

The enzymes necessary for the further conversion of D-glucuronic acid to L-ascorbic acid have been demonstrated in rat liver. For instance, a TPN-linked\* dehydrogenase present in the soluble fraction reversibly catalyzes the reduction of D-glucuronic acid to L-gulonic acid [22-24]. L-Gulonic acid is in turn converted to L-gulonolactone by a lactonase which is also present in the soluble fraction [25]. Enzymes in rat liver microsomes convert L-gulonolactone to L-ascorbic acid [26], presumably through 2-keto-L-gulonolactone as an intermediate [27].

#### BASIC DEFECT IN SCURVY

Man, other primates and the guinea pig are the only mammals known to be unable to synthesize L-ascorbic acid, and consequently they require vitamin C in their diet for the prevention of scurvy. An explanation for this has come from recent studies showing that these species are unable to convert L-gulonolactone to L-ascorbic acid. (Fig. 1.) For instance, it has been demonstrated *in vivo* that guinea pigs could not convert C<sup>14</sup> labeled D-glucuronolactone and L-gulonolactone to L-ascorbic acid; in contrast, rats converted appreciable amounts of both compounds [13]. Results which appeared almost simultaneously from two laboratories further pointed out the nature of this missing step. Lehninger and co-workers [22,28] reported no net synthesis of L-ascorbic acid from L-gulonolactone in human, monkey and guinea pig liver homogenates; whereas significant synthesis of the vitamin occurred in rat, mouse, rabbit and dog liver homogenates. Burns and co-workers [26,29] found no detectable conversion of L-gulonolactone-1-C<sup>14</sup> to labeled L-ascorbic acid in human, monkey and guinea pig liver homog-

\* DPN and TPN refer to diphosphopyridine nucleotide and triphosphopyridine nucleotide, respectively.

TABLE I  
CONVERSION OF L-GULONOLACTONE-1-C<sup>14</sup> TO L-ASCORBIC ACID IN RAT, GUINEA PIG, MONKEY AND HUMAN LIVER\*

Species	Per cent Conversion	
	Homog-enate	Micro-somes
Rat.....	8.0	10.0
Guinea pig.....	<0.05	<0.05
Monkey.....	<0.07	.....
Man.....	<0.07	.....

\* In these experiments 5.0 mg. of L-gulonolactone was incubated for 90 minutes at 37°C. under air in 5 ml. of 10 per cent homogenate (or microsomes from an equivalent amount of liver) in 0.15 M phosphate buffer (pH 7.20). L-Ascorbic acid was isolated by a carrier dilution procedure.

enates; the conversion being less than one one hundredth that obtained under the same conditions in the rat. (Table I.)

Man, monkey and guinea pig can carry out the various steps required in Figure 1 for the biosynthesis of the vitamin from D-glucose, except the conversion of L-gulonolactone to L-ascorbic acid. This reaction is presumably missing because of a gene-controlled enzyme deficiency [30]. Thus the need for vitamin C in the diet of man for the prevention of scurvy can now be considered the result of a defect in carbohydrate metabolism.

#### BIOSYNTHESIS OF L-ASCORBIC ACID IN PLANTS

Isherwood and co-workers [11] demonstrated that L-gulonolactone, L-galactonolactone, D-glucuronolactone and methyl-D-galacturonate were converted to L-ascorbic acid in cress seedlings. From their studies, they proposed two pathways for the synthesis of L-ascorbic acid in plants: one which is similar to that found in rats (Fig. 1) and the other involving D-galacturonic acid and L-galactonolactone as intermediates.

More recently Loewus and co-workers [31] have demonstrated that the major pathway for the biosynthesis of L-ascorbic acid from D-glucose in plants is completely different from that found in animals. Isotopic studies carried out in the ripening strawberry and the germinating cress seedling showed that the vitamin is synthesized from D-glucose by a fairly direct



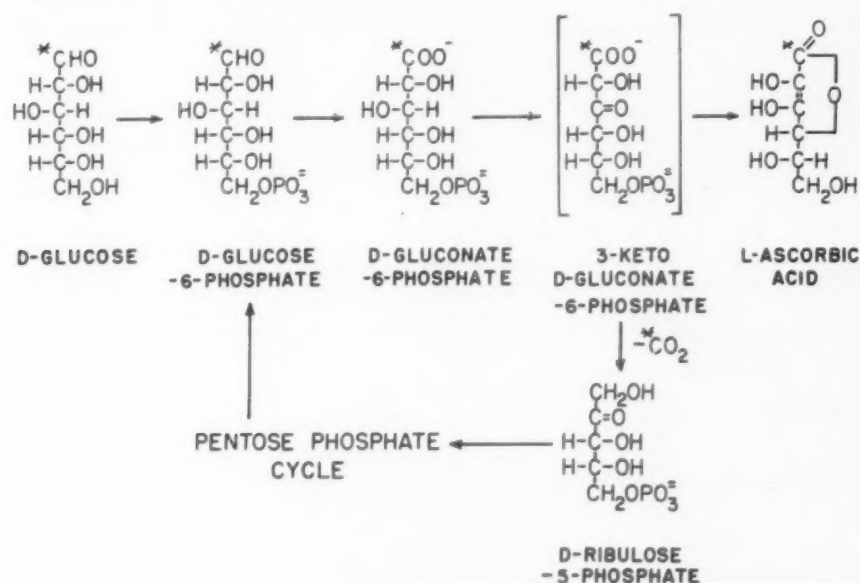


FIG. 3. Postulated pathway for the biosynthesis of L-ascorbic acid in plants involving reactions of the hexose monophosphate shunt. The asterisks denote the fate of C-1 of D-glucose in this scheme.

route in which no inversion of the carbon chain occurs. In this case, D-glucose-1-C<sup>14</sup> was converted to L-ascorbic acid labeled predominantly in C-1, and D-glucose-6-C<sup>14</sup> was converted to L-ascorbic acid labeled predominantly in C-6. It will be recalled that in the rat, C-1 of D-glucose yielded C-6 of L-ascorbic acid, and C-6 of D-glucose yielded C-1 of L-ascorbic acid. (Fig. 1.) Based on their studies with labeled D-glucose, Loewus and co-workers have postulated that the reactions of the hexose monophosphate shunt [32,33] (Fig. 3) may be involved in the synthesis of the vitamin in plants. For the conversion of 3-keto-D-gluconate-6-PO<sub>4</sub> to L-ascorbic acid, an epimerization reaction at C-5 would be required, followed by the loss of phosphate from the resulting compound and its subsequent lactonization and enolization.

The possibility that animals may also synthesize L-ascorbic acid by the scheme proposed for plants in Figure 3 appears unlikely both from the original data of King et al. with labeled glucose [8,9] and from the results of recent experiments with D-gluconate-1-C<sup>14</sup>.<sup>\*</sup> When large doses of this labeled compound were administered to rats no detectable C<sup>14</sup> was recovered in L-ascorbic acid. Since the reactions of the hexose monophosphate shunt [32,33] occur in animal tissues, it appears that animals, unlike plants, lack the ability to carry out the epimerization reaction at C-5 of the 3-keto derivative.

<sup>\*</sup> C. EVANS and J. J. BURNS. To be published.

#### FATE OF L-ASCORBIC ACID IN ANIMALS

Studies with L-ascorbic acid, labeled in various positions with C<sup>14</sup>, have shown that the vitamin is extensively oxidized to respiratory CO<sub>2</sub> in rats and guinea pigs [34-36]. In contrast, no conversion of L-ascorbic acid-1-C<sup>14</sup> to respiratory CO<sub>2</sub> was detected in man [37]. The data obtained in three human subjects with L-ascorbic acid-1-C<sup>14</sup> are compared in Table II with results found in two guinea pig experiments. L-Ascorbic acid disappears slowly in man with a half-life of

TABLE II  
C<sup>14</sup> IN EXPIRED CO<sub>2</sub> AND URINE DURING TEN-DAY PERIOD  
AFTER DOSE OF L-ASCORBIC ACID-1-C<sup>14</sup> TO MAN AND  
GUINEA PIG

Experiment	Per cent of Administered C <sup>14</sup> in *	
	CO <sub>2</sub>	Urine
Man.....	<5 <sup>a</sup>	46
Man.....	<5	45
Man.....	<5 *	36
Guinea pig.....	69	21
Guinea pig.....	85	10

<sup>a</sup> L-Ascorbic acid was administered to the human subjects intravenously in doses of 30 mg. and to guinea pigs intraperitoneally in doses of 1 to 2 mg. Less than 1 per cent of dose was eliminated in feces during a ten-day period.

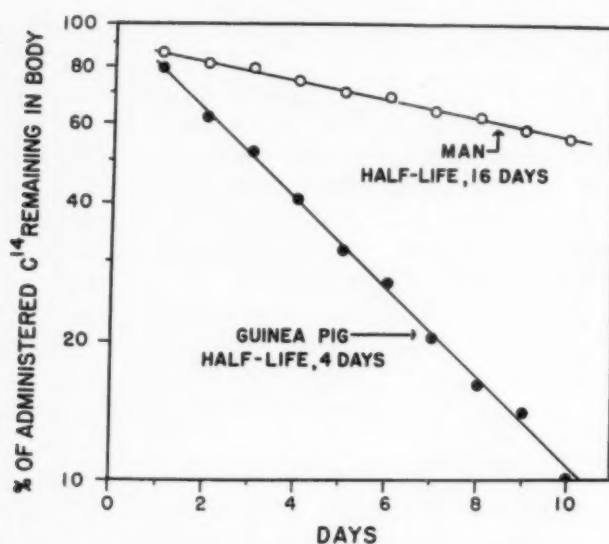


FIG. 4. Disappearance of L-ascorbic acid-1-C<sup>14</sup> after its administration to man and the guinea pig. The per cent of dose remaining in body was calculated by subtracting amount of C<sup>14</sup> excreted in urine and expired CO<sub>2</sub> during various time intervals from dose of C<sup>14</sup> administered.

about sixteen days compared to a half-life of about four days for the guinea pig. (Fig. 4.) This difference is correlated with the much longer time it takes human subjects to show signs of scurvy than observed in the guinea pig. It requires three to four months for scurvy to develop in a man on a diet free of vitamin C, while the guinea pig becomes scorbutic in about three weeks.

L-Ascorbic acid-1-C<sup>14</sup> is converted to labeled urinary oxalate in man [37], guinea pig [34] and rat [36], presumably through the intermediate formation of its oxidized products, dehydro-L-ascorbic acid and 2,3-diketo-L-gulonic acid. Although it is known that C-1 and C-2 of L-ascorbic acid contribute to oxalate formation, the specific mechanisms involved have not been elucidated. Conversion of L-ascorbic acid to oxalate may account for the major part of the endogenous urinary oxalate excreted by man [37].

#### L-ASCORBIC ACID, AN INTERMEDIATE IN CARBOHYDRATE METABOLISM

Recent studies in animals have shown that L-ascorbic acid can be metabolized back to D-glucose [38,39]. For instance, experiments in guinea pigs, conditioned for glycogenesis, showed that C<sup>14</sup>-labeled L-ascorbic acid was converted significantly to glycogen. A possible clue to the intermediates involved has come from the finding that enzymes present in guinea pig liver and

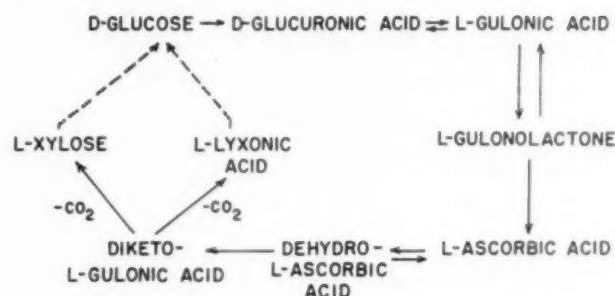
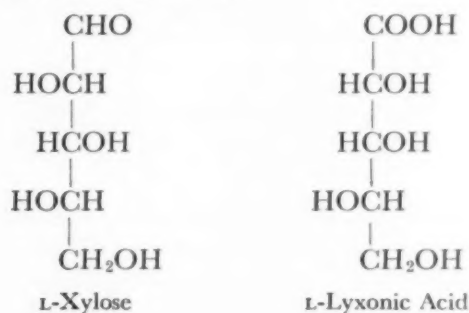


FIG. 5. L-Ascorbic acid shown as an intermediate in a cyclic pathway of carbohydrate metabolism in animals.

rat kidney decarboxylate dehydroascorbic acid with the formation of L-xylose [38] and L-lyxonic acid [40,41], respectively.



Therefore, evidence is available both for the conversion of D-glucose to L-ascorbic acid and for its transformation back to D-glucose. It now appears that L-ascorbic acid may be considered to be an intermediate in a cyclic pathway of carbohydrate metabolism, as shown in Figure 5. It should be recalled in this connection that primates and the guinea pig lack the step needed for the conversion of L-gulonolactone to L-ascorbic acid.

Loewus and co-workers [37] have recently postulated a role for L-ascorbic acid as an intermediate in carbohydrate metabolism in plants. This was suggested by the rapid incorporation of C<sup>14</sup> from L-ascorbic acid-6-C<sup>14</sup> into the carbohydrate pool of the young grape leaf. As pointed out in a previous section, D-glucose is converted to L-ascorbic acid in plants by a pathway entirely different from that given in Figure 5.

#### THE GLUCURONIC ACID PATHWAY OF GLUCOSE METABOLISM IN ANIMALS

**Over-all Pathway.** In recent years a new cyclic pathway of glucose metabolism in animals, the glucuronic acid pathway, has been elaborated, based largely on studies of the biosynthesis of L-ascorbic acid and L-xylulose [15,42,43]. This pathway, together with certain related reactions

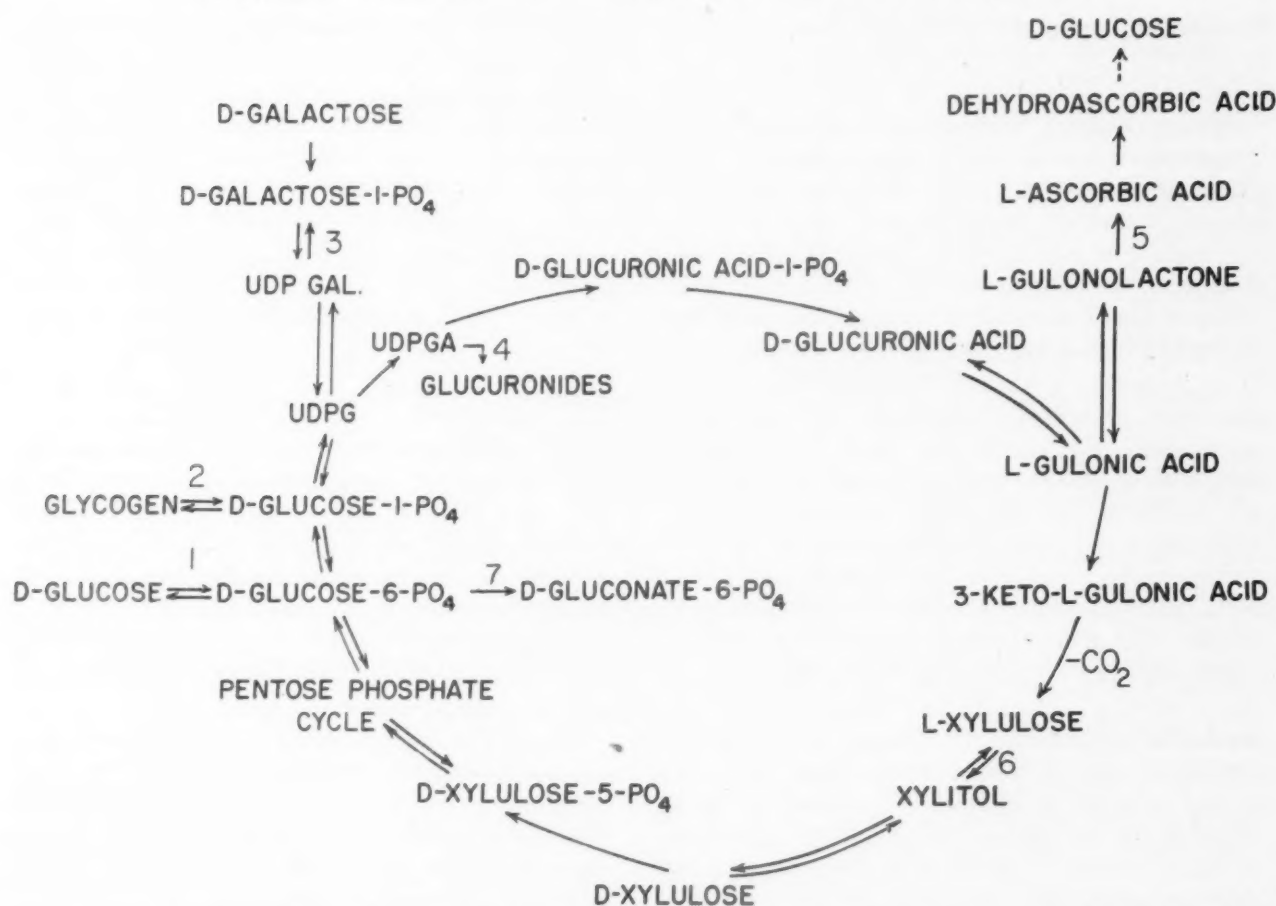


FIG. 6. The glucuronic acid pathway of glucose metabolism, shown in relation to certain other reactions of carbohydrate metabolism, to indicate the site of blocks of clinical significance. (1) Glucose-6-phosphatase is lacking in the liver of patients with a type of glycogen storage disease [62]. (2) The enzymes responsible for the branched structure of glycogen are defective or absent in another form of glycogen storage disease [63,64]. (3) The enzyme for this reaction, galactose-1-PO<sub>4</sub> uridyl transferase, is missing in patients with congenital galactosemia [65]. (4) Patients with congenital, nonobstructive, non-hemolytic jaundice exhibit a reduced rate of glucuronide formation [66]. (5) Man, monkey and guinea pigs lack this enzyme system required for the biosynthesis of L-ascorbic acid [29]. (6) The conversion of L-xylulose to xylitol is presumed to be the defect in essential pentosuria [52,53]. (7) Patients with primaquine induced hemolytic anemia or favism have a metabolic lesion in their erythrocytes associated with a deficiency of glucose-6-phosphate dehydrogenase activity [67,68].

of carbohydrate metabolism, is illustrated in Figure 6. According to this scheme D-glucose-1-PO<sub>4</sub> is oxidized to D-glucuronic acid through a mechanism involving uridine nucleotides and D-glucuronic acid-1-PO<sub>4</sub> as intermediates [17-21]. D-Glucuronic acid undergoes reduction to L-gulonic acid, which can serve as the eventual precursor of either L-ascorbic acid or L-xylulose. All animals studied can metabolize L-gulonic acid via L-xylulose, but only primates and the guinea pig lack the ability to convert L-gulonic acid to L-ascorbic acid.

Recent studies have provided evidence that 3-keto-L-gulonic acid is an intermediate in the formation of L-xylulose by a DPN-dependent enzyme in the soluble fraction of kidney [44]. Touster and co-workers [45,46] have demon-

strated an enzyme system in liver capable of reversibly reducing both L-xylulose and D-xylulose to a common intermediate, xylitol, thereby providing a mechanism for the interconversion of the stereoisomers of this ketopentose. The subsequent finding by Hickman and Ashwell [47] of a specific liver kinase capable of forming D-xylulose-5-PO<sub>4</sub> from the free sugar indicated that mammalian tissues possess the complete enzymatic structure necessary to carry out the conversion of L-xylulose to D-glucose via the pentose cycle [48,49]. Evidence for the occurrence of this cyclic pathway in the intact rat and guinea pig has come from recent findings that labeled D-glucuronolactone [50], L-gulonolactone [51] and xylitol [43] are converted to liver glycogen in accordance with this scheme.



Studies employing labeled D-glucuronolactone in subjects with essential pentosuria have provided evidence for these reactions in man [52,53].

**Effect of Drugs.** Various drugs markedly increase the rate at which glucose enters the glucuronic acid pathway. For example, the administration of barbitol and chloretone to rats leads to a marked increase in the conversion of glucose to D-glucuronic acid [15], L-gulonic acid [14] and L-ascorbic acid [16]. This stimulatory effect on L-ascorbic acid biosynthesis in the rat is shown by a variety of foreign compounds possessing completely unrelated chemical and pharmacological properties [54,55]. Included among these are the hypnotic drugs: Chloretone and barbitol; the analgesics: aminopyrine and antipyrine; the muscle relaxants: arphenadrine and meprobamate; and the carcinogenic hydrocarbons: 3-methylcholanthrene and 3,4-benzpyrene. It is of considerable interest that in 1935 Enklewitz and Lasker [56] found that two of these drugs, aminopyrine and antipyrine, markedly increased the urinary excretion of L-xylulose in patients with pentosuria. It is now possible to explain their observation in terms of the scheme in Figure 6. Administration of these drugs would be expected to increase the formation of L-xylulose from D-glucose. Since the pentosuric patient is not able to metabolize L-xylulose, the pentose would consequently be excreted in urine.

The mechanisms by which drugs increase the biosynthesis of L-ascorbic acid from D-glucose is not known. Metabolism or conjugation of the drug is not required, since barbitol, one of the most potent of these drugs, is excreted unchanged in urine [15]. It is possible that this effect of drugs on glucose metabolism may represent a new adaptive response on the part of the body to foreign compounds. This is suggested from the observation that those compounds which are potent in stimulating the synthesis of L-ascorbic acid, such as barbitol, Chloretone,<sup>®</sup> and the previously mentioned carcinogenic hydrocarbons, also increase the activity of liver microsomal enzymes which metabolize various foreign compounds [55,57].

**Metabolism of Inositol.** Recent studies have shown that the glucuronic acid pathway may be an important route for the metabolism of inositol in the rat. The first indication of this came from the observation that inositol is converted by kidney enzymes to a racemic mixture of D,L-glucuronic acid [58]. Subsequent

experiments with labeled inositol indicate that the compound is transformed *in vivo* to D-glucuronic acid and L-gulonic acid [59], and that it yields glucose labeled in accordance with the predictions of the scheme given in Figure 6 [60,61]. However, no conversion of labeled inositol to L-ascorbic acid was detected [59], which has been explained in terms of the distribution of enzymes involved in the catabolism of inositol and in the biosynthesis of L-ascorbic acid.

#### CONCLUSIONS

L-Ascorbic acid is synthesized in rats as follows: D-Glucose → D-glucuronic acid → L-gulonic acid → L-gulonolactone → L-ascorbic acid. However, man, monkey and guinea pig lack the liver enzyme system required for the conversion of L-gulonolactone to L-ascorbic acid. It is this missing step which makes necessary the inclusion of vitamin C in the diet for the prevention of scurvy.

Plants synthesize L-ascorbic acid from D-glucose by a pathway altogether different from that in the rat. The reactions of the hexose monophosphate shunt have been postulated to be involved in the plant pathway.

There are marked differences in the metabolism of L-ascorbic acid in man and in the guinea pig, two species that require L-ascorbic acid as a vitamin in their diet. For example, the conversion of the carboxyl carbon of L-ascorbic acid to CO<sub>2</sub> is a main route of its metabolism in guinea pig, whereas this reaction does not occur to any detectable extent in human subjects. In addition, L-ascorbic acid is metabolized at a much slower rate in man than in the guinea pig. This difference can explain why a much longer time is required for scurvy to develop in man on a vitamin C-free diet than in the guinea pig.

Studies on the biosynthesis of L-ascorbic acid have played an important role in uncovering a new route of glucose metabolism in animals, the glucuronic acid pathway. According to this scheme, D-glucose is metabolized via D-glucuronic acid, L-gulonic acid, L-xylulose, D-xylulose and the pentose phosphate cycle. Of considerable interest is the observation that various drugs, possessing completely unrelated chemical and pharmacological properties, markedly increase the amount of glucose metabolized via this pathway. It is possible that this effect may represent a new adaptive response on the part of the body to foreign compounds.

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# Mucopolysaccharide Metabolism\*

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THIS review will stress the metabolism of mucopolysaccharides and their carbohydrate constituents, with particular emphasis on *in vitro* or enzymatic studies. Due to limitations of space, only a few of the numerous publications in this field will be considered. †

## NATURE AND DISTRIBUTION OF THE MUCOPOLYSACCHARIDES

Mucopolysaccharides are classified as part of a group of compounds frequently called the "mucoid substances." Chemical information concerning the nature of these substances is so limited that numerous systems of nomenclature have appeared [12] and none of them has been generally accepted. Table I indicates the general properties of some of the mucoid substances. In general, the mucopolysaccharides may be defined as polysaccharides which usually contain

† A number of reviews are mentioned in the text. In addition, the following are concerned with aspects of connective tissue biochemistry: collagen and fibrous proteins [1,2]; connective tissue [3]; polysaccharides, mucopolysaccharides, mucolipides, etc., [4]; bacterial polysaccharides [5]; virus enzymes [6]; amino sugars [7]; mucoid substances [8]; physiology of connective tissue [9]; connective tissue diseases [10]; the blood group substances [11].

NOTE: The following abbreviations are used throughout: UDP = uridine diphosphate; GDP = guanosine diphosphate; ATP = adenosine triphosphate; CoA = Coenzyme A; AcCoA = acetyl coenzyme A; G = D-glucose; G-1-P = D-glucose-1-phosphate; Gal = D-galactose; F = D-fructose; Fu = L-fucose; Rh = L-rhamnose; M = D-mannose; Gm = D-glucosamine = 2-deoxy-2-amino-D-glucose; Galm = D-galactosamine = 2-deoxy-2-amino-D-galactose; Mm = D-mannosamine = 2-deoxy-2-amino-D-mannose; N-AcGm = N-acetylglucosamine; N-AcGalm = N-acetylgalactosamine; N-AcMm = N-acetylmannosamine; GA = D-glucuronic acid; GalA = D-galacturonic acid; NANA = N-acetylneuraminic acid; HA = hyaluronic acid; CSA = chondroitin sulfate; APS = adenosine-5'-phosphosulfate; PAPS = adenosine-3'-phosphate-5'-phosphosulfate. Other abbreviations are noted at appropriate places in the text.

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hexosamine and are associated with protein, but which can be separated from protein by relatively mild technics. According to this definition, certain bacterial and fungal polysaccharides may be considered mucopolysac-

TABLE I  
NOMENCLATURE\*

% Carbohydrate	Names	Examples
0.....	Protein	Insulin
Trace-15....	Glycoprotein, protein	Albumins, globulins, collagen
10-85.....	Mucoproteins	Orosomucoid, blood group substances, urinary mucoprotein
65-100.....	Mucopolysaccharides	Hyaluronic acid, chondroitin sulfates, chitin, heparin
100.....	Polysaccharides	Cellulose, hemicelluloses

\* Only high molecular weight compounds which contain carbohydrate and/or amino acid are considered. (1) The compounds are classified on the basis of their relative quantities of amino acid and carbohydrate. However, it should be stressed that there are no sharp lines of demarcation, the groups therefore overlap, and the values used are strictly arbitrary numbers which will probably change as more precise information is gathered. (2) Few proteins are carbohydrate-free. (3) While the mucopolysaccharides can be isolated as high molecular substances free of protein or peptide residues, separation of amino acids from carbohydrate residues in the glycoprotein and mucoprotein classes, requires extensive degradation to low molecular weight compounds. However, the mucopolysaccharides may be combined with proteins *in vivo*. For example, chondroitin sulfate of cartilage exists in complex with protein although the nature of the bond is unknown. In other cases, notably hyaluronic acid and heparin, it is not yet clear whether the polysaccharides exist in complex with protein by salt or hydrogen bonding or by some labile covalent bond. (4) The present concept of glycoprotein and mucoprotein structure suggests that these substances contain oligosaccharides and peptides which are covalently bonded to yield high molecular weight compounds. The nature of these bonds is unknown. (5) The difference between the glycoproteins and mucoproteins resides in their chemical and physical properties. Thus the mucoproteins generally have a higher carbohydrate content and are more stable to a variety of agents such as heat and protein precipitants [17]. (6) The carbohydrate constituents of the substances in this table vary considerably, but with few exceptions, the "mucoid" substances contain hexosamine.

Certain "mucoid" substances also contain lipide, but the situation here is even more confusing than that already described. A comprehensive review on brain lipides (LEBARON, F. N. and FOLCH, H. *Physiol. Rev.*, 37: 539, 1957) indicates the complexity of the problem. Rosenberg and Chargaff (*J. Biol. Chem.* 232: 1031 1958) have recently proposed a definition for mucolipides; they are "soluble in water, but also in organic solvents, contain fatty acid, a sphingosine-like base, a hexose, also amino sugar, sometimes amino acid, and most significantly, sialic acid or a related substance." It should be noted these substances differ from many of the lipopolysaccharides (STACEY, M. *Advances Carbohydrate Chem.*, 2: 162, 1946).

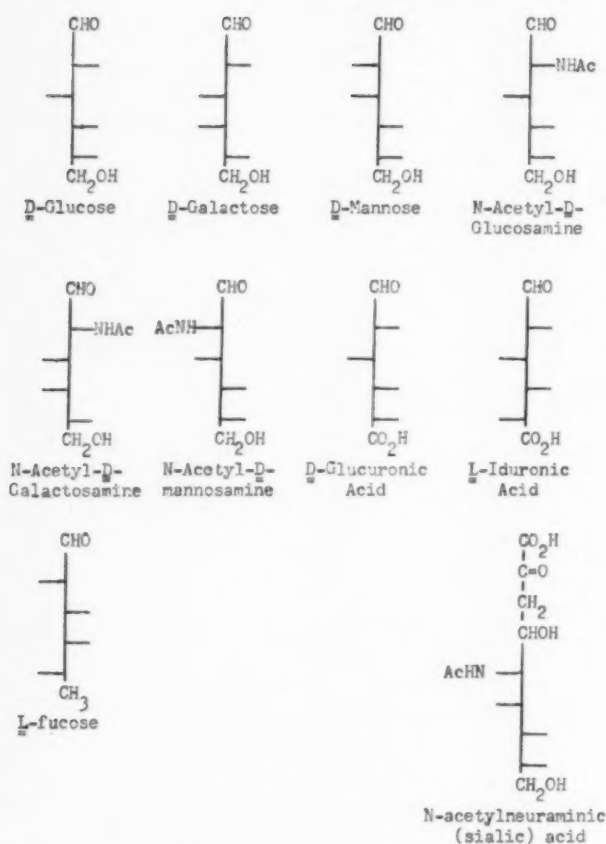


FIG. 1. Carbohydrate constituents of animal mucoid substances.

carides in addition to those isolated from animal sources.

Connective tissue is particularly rich in the mucoid substances, and is presumed to be the ultimate source of mucopolysaccharides in the vertebrate. The following polysaccharides have been isolated and characterized: heparin, heparitin sulfate [13],\* chondroitin sulfates A, B and C, hyaluronic acid, keratosulfate and chondroitin. Many of these substances have been isolated and characterized by Meyer and his associates, using classic chemical fractionation technics. It seems reasonable to assume, however, that further studies on connective tissue will show the presence of additional mucopolysaccharides. The tissue sources for some of the mucopolysaccharides are presented in Table II, and the carbohydrate constituents of these polymers and related substances are given in Table III and Figure 1.

It may be noted here that the isolation and

\* There is no generally accepted nomenclature for the various mucopolysaccharides. For example, chondroitin sulfate B has also been designated  $\beta$ -heparin, dermoitin sulfuric acid, and gastroitin sulfuric acid.

TABLE II  
DISTRIBUTION OF SOME MUCOPOLYSACCHARIDES\*

Tissue	CSA					Chon-droitin	Others
	HA	A	C	B	KS		
Vitreous humor.....	+	..	..	..	..	..	..
Synovial fluid.....	+	..	..	..	..	..	..
Fowl tumors.....	+	..	..	..	..	..	..
Liposarcoma.....	+	..	..	..	..	..	..
Cartilage.....	..	+	+	..	+	..	..
Adult bone.....	..	+	..	..	..	..	..
Chondrosarcoma.....	..	+	+	..	..	..	..
Chordoma.....	..	+	+	..	..	..	..
Umbilical cord.....	+	..	+	..	..	..	..
Fibroblasts (tissue culture)...	+	..	+	..	..	..	..
Electric eel.....	+	..	+	..	..	..	..
Pig skin.....	+	..	+	..	..	..	..
Ligamentum nuchae.....	+	+	+	+	..	..	..
Tendon.....	+	+	+	..	..	..	..
Heart valve.....	+	+	+	+	..	..	..
Cornea.....	..	+	+	..	+	+	..
Calf bone.....	..	+	+	..	+	..	+
Aorta.....	+	+	..	+	..	..	+

\* See Meyer et al. [14] for quantitative relationships. This table is not intended to be complete. For example, HA occurs in tissue and tissue exudates other than those indicated (e.g., human mesothelioma). Further, other mucopolysaccharides, such as heparin, occur in some of the tissues referred to in the table. Minor components may be present in all tissues, but would not be detected by the fractionation procedures.

HA = hyaluronic acid; CSA = chondroitin sulfate; KS = kerato-sulfate; "others" include unidentified fractions.

TABLE III  
COMPOSITION OF SOME MUCOPOLYSACCHARIDES

Mucopolysaccharides	Constituents*
Chitin.....	AcGm
Hyaluronic acid.....	AcGm, GA
Chondroitin.....	AcGalm, GA, sulfate (?)
Chondroitin sulfates:	
A.....	AcGalm, GA, sulfate
B ( $\beta$ -heparin).....	AcGalm, L-iduronic, sulfate
C.....	AcGalm, GA, sulfate
Keratosulfate.....	AcGm, Gal, sulfate
Heparin.....	Gm, GA, sulfate
Heparitin sulfate.....	Gm, GA, acetate, sulfate

\* AcGm = N-acetyl-D-glucosamine; AcGalm = N-acetyl-D-galactosamine; GA = D-glucuronic acid; Gal = D-galactose.

characterization of the mucopolysaccharides is a complex task, particularly since the homogeneity of an isolated fraction is difficult to determine. Thus only one chondroitin sulfate was originally recognized [15], then three [14], and now it is suggested that chondroitin sulfate B may consist of two fractions [16]. Obviously, this problem of isolation of homogeneous substances greatly enhances the difficulties of metabolic studies on the mucopolysaccharides.

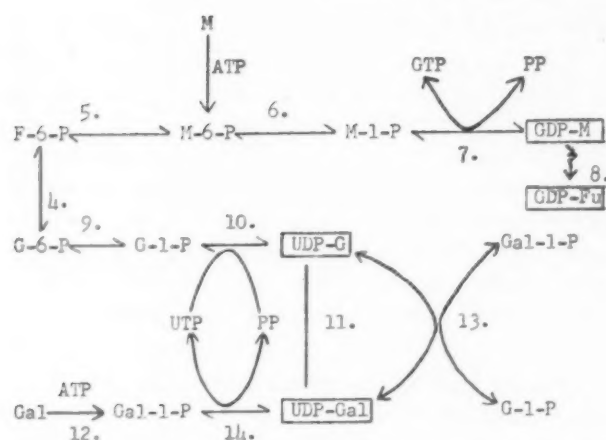


FIG. 2. Galactose, glucose, mannose and fucose metabolism. F-6-P = Fructose-6-phosphate; Gal = galactose; G = glucose; M = mannose; Fu = fucose; GDP = guanosine diphosphate; UDP = uridine diphosphate.

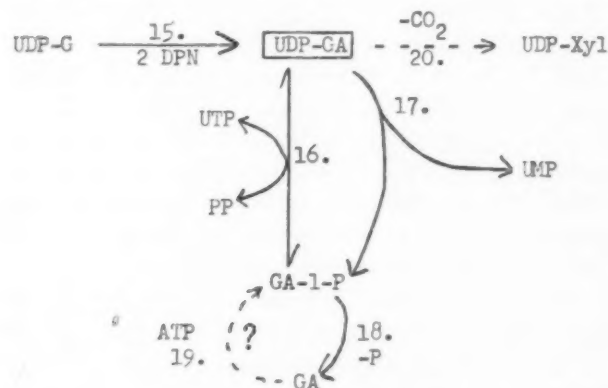


FIG. 3. Glucuronic acid metabolism. G = glucose; GA = D-glucuronic acid; Xyl = xylose; UDP = uridine diphosphate.

#### MONOSACCHARIDE METABOLISM

Although the biosynthesis of the mucopolysaccharides is poorly understood, there is a good deal of information available concerning the biosynthesis of some of the monosaccharide units of these polymers. These pathways are summarized in Figures 2 through 5. Certain of these reactions will be considered subsequently. The metabolism of uronic acid, ascorbic acid and galactose are reviewed in detail elsewhere in this symposium.

**Sugar Nucleotides.** Recent work in a number of laboratories has demonstrated that the sugar nucleotides are of primary importance in the intermediary metabolism of the monosaccharides. These nucleotides were discovered independently by two groups of investigators [17,18]. In addition to discovering these compounds originally, Leloir and his colleagues

MAY, 1959

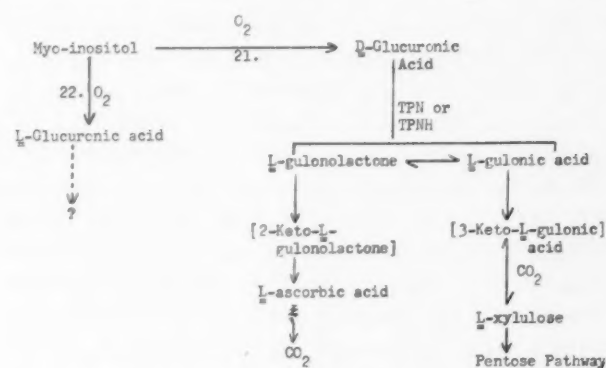


FIG. 4. Glucuronic acid metabolism.

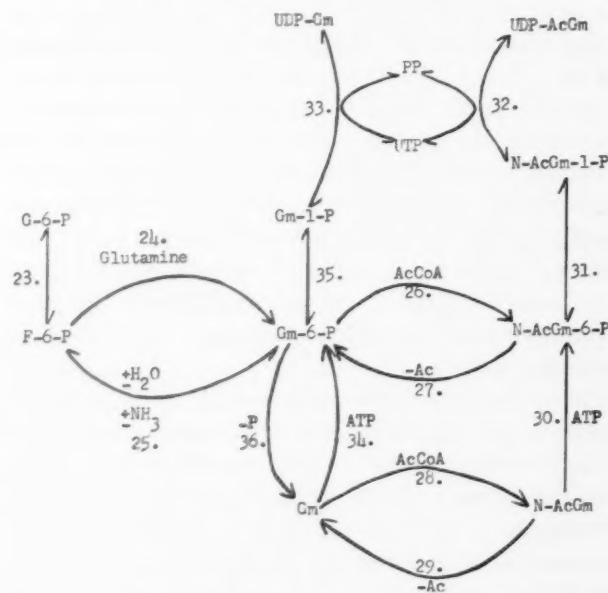
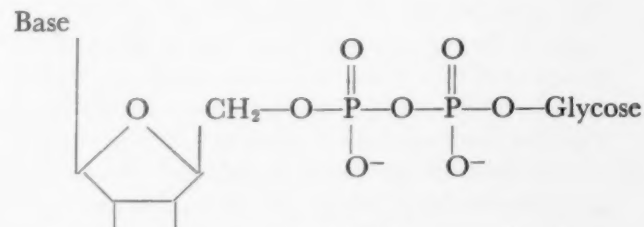


FIG. 5. Glucosamine metabolism. AcCoA = acetyl Coenzyme A.

[19] have defined many of the enzymatic reactions in which these substances participate. The compounds have the general formula indicated:



Base = Purine or pyrimidine.

Glycose = Sugar residue.

Guanosine is present in two of the sugar nucleotides; guanosine diphosphate mannose (GDP-M) [20] and guanosine diphosphate fucose (GDP-Fu), presumably L-fucose (Fu) [21,22]. The list of uridine sugar nucleotides

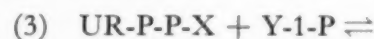
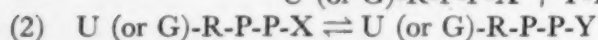
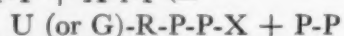
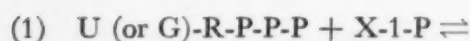


increases annually and now includes derivatives of the following sugars; D-glucose (G), D-galactose (Gal), D-glucosamine (Gm), D-xylose (Xyl), L-arabinose (Arab), muramic acid, muramic acid peptides, N-acetyl-D-glucosamine (N-AcGm or AcGm), N-acetyl-D-galactosamine (N-AcGalm or AcGalm), D-glucuronic acid (GA), D-galacturonic acid (GalA), a phosphate ester of UDP-AcGm and a sulfate ester of UDP-AcGalm. The chemistry of these compounds and other similar substances has recently been reviewed [23], and is discussed here only with respect to their metabolism.

All cells and tissues thus far examined yielded sugar nucleotides, although problems of isolation and characterization have precluded accurate studies on the quantitative and qualitative relationships of different nucleotides from a single source. Unidentified nucleotide peaks frequently have been isolated by ion exchange chromatography. It is of interest to note that sheep milk is a particularly rich source for these materials [22,24]; the non-protein organic phosphate of the milk consists primarily of these compounds.

It should perhaps be noted that only a few of the sugars contained in the sugar nucleotides have been completely characterized. The available evidence suggests, but does not yet prove, that: (1) all the naturally occurring sugar nucleotides are of the D- configuration excepting L-fucose and L-arabinose; (2) the sugars exist in the pyranose ring form; and (3) all the glycosidic bonds are of the  $\alpha$  configuration with the exception again of the fucose and arabinose derivatives. As is discussed later, nucleotides are substrates for the formation of polysaccharides which may be either of the  $\alpha$  or the  $\beta$  configuration. Closer attention must therefore be paid to the structure of the sugar nucleotides which are used in these experiments. An illustration of this point is the fact that the usual glucose-1-phosphate ester is  $\alpha$ -D-glucopyranose in structure, but the corresponding  $\beta$  isomer does occur [25] and it is therefore possible that the corresponding  $\beta$  isomer of UDP-glucose may also exist.

**Biosynthesis of Sugar Nucleotides.** Enzymatic studies have demonstrated that the sugar nucleotides can arise by one of the following reactions:



U = uracil; G = guanine; X and Y = glucose residues

For example, the sugar phosphates such as  $\alpha$ -G-1-P and UTP are converted to UDP-G and pyrophosphate by enzymes called pyrophosphorylases (reaction 1). Reaction 2 is a generalized formulation for the conversion of one sugar nucleotide to another by processes involving either oxidation (UDP-G  $\rightarrow$  UDP-GA in the presence of DPN and the appropriate dehydrogenase), reduction (GDP-M  $\rightarrow$  GDP-Fu in the presence of TPNH), or epimerization (UDP-G  $\rightleftharpoons$  UDP-Gal). Epimerization reactions which do not involve sugar nucleotides are discussed elsewhere in this symposium. Reaction 3 has been described only in the case of galactose-1-phosphate in which there is a transfer of the UMP residue from UDP-G to the galactose-1-phosphate with the formation of UDP-Gal.

*Metabolism of the Aldohexoses and the 6-Deoxyaldohexoses.* The reactions outlined in Figure 2 show the conversion of G-6-P, galactose and mannose to the corresponding nucleotides of these compounds and of L-fucose.

In studies of this kind, one is always faced with the problem of enzyme specificity. Thus, is the UDP-G pyrophosphorylase (reaction 10) of animal, bacterial and plant cells a specific enzyme, or is it also responsible for the pyrophosphorolysis of other sugar nucleotides? This question has been answered by experiments with crude mung bean extracts [26] which catalyze the pyrophosphorolysis of several uridine nucleotides (reaction 1). It was shown [27] that the purified enzyme from mung bean extract was specific for UDP-G. Further, a preliminary report [28] indicates that three pyrophosphorylases from yeast could be separated by suitable fractionation procedures and were specific for UDP-AcGm, UDP-G and GDP-M. The metabolism of galactose has been reviewed elsewhere [29,30] and is also discussed in another paper of the present symposium. These studies encompass reactions 10 through 14.

A number of studies with labelled glucose and bacterial systems indicated that the D-glucose carbon chain was incorporated directly into that of the L-fucose carbon chain [31-34]. A direct conversion of this sort requires epimerizations at carbons 2, 3 and 5, and reduction at carbon 6. More precise information on the intermediates

in the over-all conversion was obtained following the isolation of GDP-Fu from sheep milk [22] and *Aerobacter aerogenes* [27]. Ginsburg [35] then reported the enzymatic conversion of GDP-M to GDP-Fu in the presence of extracts from *A. aerogenes* and TPNH. Obviously, several steps are involved in this process; the exact steps are not yet known.

**Uronic Acid and Pentose Metabolism.** The naturally occurring uronic acids are D-glucuronic, D-galacturonic, L-iduronic, D-mannuronic and L-guluronic. Thus far, only D-glucuronic and L-iduronic acids have been found as constituents of animal mucopolysaccharides, although the presence of D-galacturonic has been claimed. At an enzymatic level, there is a considerable body of information concerning the metabolism of D-glucuronic acid and some information about D-galacturonic acid; there is almost no information on the metabolism of the other uronic acids.

The two major pathways of glucuronic acid metabolism involve either the phosphorylated compounds shown in Figure 3 or the free sugars shown in Figure 4. As already indicated, UDP-G is oxidized to UDP-GA by a dehydrogenase (reaction 15) which occurs in animal, plant and bacterial cells [36,37]. The reaction appears to be irreversible. A pyrophosphorylase (reaction 16) also has been described in plant extracts but has not yet been reported in animal tissues [36]. If the pyrophosphorylase serves as a mechanism for the synthesis of UDP-GA, then the enzymatic synthesis GA-1-P must be considered; neither a kinase (reaction 19) nor a dehydrogenase which will act on G-1-P to yield GA-1-P is known. Rat kidney hydrolyzes UDP-GA to GA via reactions 17 and 18 [38]. The synthesis of UDP-GalA, UDP-Arab and UDP-Xyl (reaction 20 and others) has been reported, utilizing plant extracts [39,40]. While the mechanism for reaction 20 has not been clarified, the formation of pentose nucleotides from uronic acid nucleotides and ultimately from hexose nucleotides confirms an old suggestion that pentose in plant tissue arises by decarboxylation of uronic acid which in turn comes from hexose.

A completely different mechanism for the formation of D-glucuronic acid is the oxidation of myo-inositol in the presence of rat kidney extracts [47]. It is surprising to note that, in addition to D-glucuronic acid, L-glucuronic acid was also produced (reactions 21 and 22). The enzyme systems responsible for the oxidations

were separated [42,43], and the one which produces the D isomer has been purified. Nothing is known concerning the further metabolism of L-GA, but the fact that it is produced in quantity equal to D-GA suggests its importance. The further metabolism of free D-GA to L-ascorbic acid, which ultimately is converted to CO<sub>2</sub>, or to L-xylulose, which ultimately enters the pentose pathway, is outlined in Figure 4 and is discussed in detail elsewhere in this symposium. It may be of interest to note that the lactones apparently are involved in the formation of ascorbic acid, while the free acids are involved in pentose formation.

**Hexosamines.** The "mucoid substances" contain D-glucosamine (Gm) and/or D-galactosamine (Galm), generally as their corresponding N-acetyl derivatives (N-AcGm and N-AcGalm). In addition, the sialic acids contain D-mannosamine as the hexosamine moiety. The metabolism of Gm and N-AcGm has been investigated more thoroughly than the related compounds and the known enzymatic pathways are indicated in Figure 5. *In vivo* studies with labelled glucose indicated that the carbon chain of D-glucose was converted intact to that of Gm [44-48]. The nitrogen atom of Gm was shown to be derived from the amide group of L-glutamine [49-51]. The first cell-free experiments on Gm formation [52] with extracts of *Neurospora crassa* suggested that hexose-P and glutamine yielded a hexosamine, presumed to be Gm-6-P (reactions 23 and 24). In further studies [53] the contaminating phosphohexoseisomerase (reaction 23) was removed from the protein fraction, and it was demonstrated that fructose-6-phosphate (F-6-P) rather than G-6-P was involved in the synthesis, as indicated in reaction 24. A preliminary communication [54] reports that enzyme systems from microbial and mammalian cells were compared, and it was concluded that F-6-P is the required substrate in all cases tested. The enzyme does not require any added cofactor such as ATP, and there is no information on the mechanism of this synthesis which involves the transfer of the amide group of glutamine to F-6-P to produce Gm-6-P and glutamic acid. The reaction appears to be irreversible.

As originally demonstrated by Lutwak-Mann [55], mammalian tissue slices and bacteria utilize Gm, Galm, and their respective N-acetyl derivatives. Apparently the first step in this utilization is phosphorylation of these compounds (reactions 30 and 34) to yield the respec-



tive 6-phosphate esters for the glucosamine derivatives and the 1-phosphate esters for the galactosamine derivatives [56].

Early observations [57-59] indicated that Gm-6-P was converted to  $\text{NH}_3$  and a hexose-P by extracts obtained from bacteria and rat brain. Subsequently [60-63], it was shown that the products were F-6-P and  $\text{NH}_3$ . The enzymes from *Escherichia coli* and pig kidney have been purified and compared [64], and it was concluded that both enzymes acted in a similar manner (reaction 25). The reaction was also shown to be slightly reversible, equilibrium greatly favoring the formation of F-6-P and  $\text{NH}_3$ . Crude pig kidney extracts also catalyzed the conversion of N-AcGm-6-P to F-6-P,  $\text{NH}_3$  and acetate. It is presumed that this sequence occurs via reactions 27 and 25, the first step being hydrolysis or deacetylation of N-AcGm-6-P. A similar deacetylase has been described in bacteria [65], as indicated in reaction 29. It is perhaps of interest to note that two pathways now exist for the formation of Gm-6-P from G-6-P via F-6-P; one is reaction 24, which requires glutamine and is irreversible, the other is reaction 25, which requires  $\text{NH}_3$  and is reversible. The equilibrium of reaction 25 can be pulled towards the right (i.e., the formation of Gm-6-P) by coupling it with an enzyme which acetylates Gm-6-P in the presence of acetyl coenzyme A, by reaction 26 [66]. The acetylating enzyme is widely distributed in nature and has been demonstrated with extracts from human liver. Initially, it was thought that Gm itself was acetylated via reaction 28 to N-AcGm. However, it has since been demonstrated that this is another case of enzymatic non-specificity. The present belief is that reaction 28 is not physiologically significant, but that acetylation occurs only with Gm-6-P and the acetylating enzyme.

A number of phosphatases hydrolyze Gm-6-P to Gm (reaction 36). The conversion of the 6-phosphate esters to the corresponding 1-phosphate esters is indicated in reactions 35 and 31. Reaction 35 is catalyzed by crystalline phosphoglucomutase [67] but the amount of enzyme required for this conversion is considerably greater than that for the comparable conversion of G-6-P to G-1-P. The mutase which acts on the N-acetyl derivatives, reaction 31, appears to be more specific [68]. Finally, the 1-phosphate esters can be converted to the uridine nucleotides via pyrophosphorylases, as indicated in reactions 32 and 33. It is not yet known whether UDP-Gm is

a natural substance, despite the enzymatic synthesis of this material [69]. This compound may be of interest in heparin synthesis since heparin is one of the two known mucopolysaccharides in which the amino group is not acetylated but N-sulfated.

UDP-AcGm can serve as the source for the synthesis of two other amino sugars. Thus rat liver extracts convert UDP-AcGm to free N-acetyl-D-mannosamine [70]. Presumably, the latter compound is then involved in the biosynthesis of one of the sialic acids. In this "epimerization," the acetylamino group at C-2 appears to be the site of enzymatic action, which represents a unique reaction in the biochemical literature; the mechanism for this reaction is not yet known. While UDP-AcGalm has been isolated from liver, although not separated from UDP-AcGm, little is known about its biosynthesis. By analogy with the interconversion of UDP-G and UDP-Gal, epimerization at C-4 of UDP-AcGm would yield UDP-AcGalm. In fact, preparations of the UDP-G-4-epimerase do catalyze the epimerization of UDP-AcGm to UDP-AcGalm.\* Whether this represents enzymatic non-specificity or the presence of two specific enzymes in the protein preparations remains to be determined.

The present information on galactosamine metabolism is too skimpy to draw any final conclusions, but suggests that the metabolism of this sugar is analogous to that of galactose, at least insofar as it involves only the 1-phosphate esters, and apparently is formed from the corresponding glucose derivative. On the other hand, there are two pieces of information which suggest that perhaps the Galm-6-P esters may be metabolically active. Thus a tentative report [71] suggests the presence of an acid-stable phosphate ester of Galm in cartilage, probably not the 1-phosphate. Secondly, chemically synthesized Galm-6-P was N-acetylated by a purified enzyme previously considered to be specific for Gm-6-P [72], i.e., reaction 26. Further work in these areas is necessary to determine whether or not Galm-6-P is actually an intermediary metabolite.

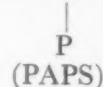
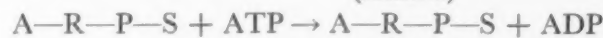
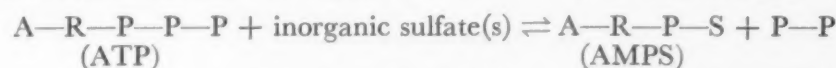
Even less is known about the metabolism of mannosamine (Mm) and N-acetylmannosamine (N-AcMm). This lack of information is due to the fact that the compound has only been recently recognized as a naturally occurring substance [73]. The only two enzymatic reactions in which Mm derivatives are known to

\* Dr. Frank Maley, private communication.



participate are: (1)  $\text{UDP-AcGm} \rightarrow \text{AcMm} + \text{UMP} + \text{P}$  [70]; (2) the enzymatic synthesis of sialic acid [73]  $\text{N-AcMm} + \text{pyruvate} \rightleftharpoons \text{N-acetylneuraminic acid (sialic acid)}$ . The latter reaction is of the aldolase type, reversible, and is specific for N-AcMm.

**Sulfate Activation and Transfer.** Many of the mucopolysaccharides contain organic or ester sulfate. Generally, the ester group is formed between the sulfate radical and one of the hydroxyl groups of the carbohydrate moieties. An exception to this rule is heparin, of which one sulfate radical per disaccharide unit is bound to the amino group forming a sulfamic acid derivative. In the few cases in which the mucopolysaccharide structures have been established, sulfate was demonstrated to be present on the hexosamine moieties at either C-4 or C-6. Enzymatic studies on the sulfate problem have been reviewed by Lipmann [74]. The following reactions are required for activation and transfer of sulfate:



A = adenine; R = ribose; P-P = pyrophosphate; ROH = sulfate acceptor

The conversion of inorganic sulfate to "active" sulfate (PAPS) has been demonstrated in a variety of tissues [75-87]. While the known sulfate acceptors (ROH) include a host of phenols and steroids, there has been no clear demonstration of sulfate transfer to a *known* carbohydrate derivative. Thus the carbohydrate acceptor for the sulfate radical may possibly be the free sugar or the phosphorylated, nucleotide, or even polysaccharide, derivatives. The interesting synthesis of chondroitin sulfate from inorganic sulfate by cartilage extracts will be discussed subsequently.

#### ENZYMATIC SYNTHESIS OF THE GLYCOSIDIC BOND AND POLYSACCHARIDE FORMATION

The mucopolysaccharides, like other polysaccharides, are polymers of monosaccharide units. A general consideration of polysaccharide synthesis involves the following questions: (1) What is the source of energy for synthesis? (2) Just as the peptide bond functions in maintenance of the protein structure, so the glycosidic bond serves as the primary structural unit in polysaccharides—how is the glycosidic bond

formed? (3) What types of isomerism are found in the polysaccharides?

The last problem will be considered first. The polysaccharides are either homopolysaccharides (i.e., containing a single glucose unit such as glucose in the cases of glycogen, cellulose and starch) or heteropolysaccharides (i.e., containing two or more glucose units, such as is the case in all the mucopolysaccharides with the exception of chitin). Polysaccharides can be straight-chained, which appears to be the case with most of the mucopolysaccharides, or branched, as is the case with glycogen and a large number of complex plant polysaccharides. The repeating units can be attached at a single position such as 1 → 4 in cellulose, chitin and amylose, or there can be linkages to other positions, as in glycogen which contains both the 1 → 4 and 1 → 6 type of glycosidic bond. There may be a simple repeating unit, as in hyaluronic acid (the repeating unit being a disaccharide containing acetyl-

glucosamine and glucuronic acid), or the problem may be much more complex with no apparent simple repeating unit, as in the case of the plant gums. The glycosidic bond may be  $\alpha$ , as in glycogen and starch, or  $\beta$ , as appears to be the situation in cellulose and in the mucopolysaccharides thus far investigated with the exception of heparin. The sugar moieties may have the pyranose configuration, which appears to be true in the mucopolysaccharides, or the furanose configuration, which occurs in some of the complex plant polysaccharides. This brief account suggests some of the complexities of polysaccharide isomerism, but space limitations do not allow further discussion of these points. It is to be noted that no consideration has been given to molecular size since the definition assumed here is that a polysaccharide is a single entity if it is non-dialyzable despite the fact that it may well be inhomogeneous with respect to molecular size.

The mechanism of formation of the glycosidic bond is obviously of prime interest. Three known biochemical mechanisms exist for the synthesis of disaccharides and polysaccharides. These

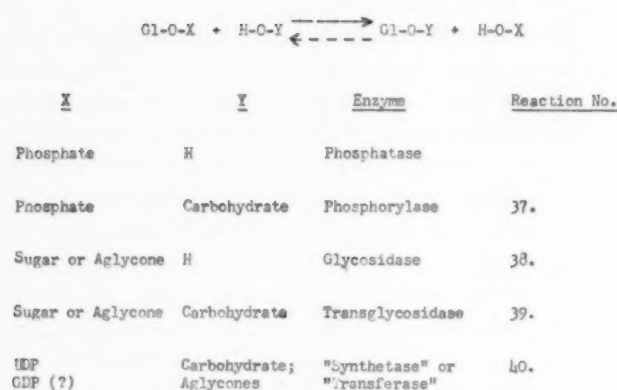


FIG. 6. Glycosyl transfers and related reactions. Gl = glycosyl residue.

general mechanisms are presented in Figure 6 along with related reactions. Glycosides can be synthesized by phosphorylases (reaction 37), transglycosidases (reaction 39), and "synthetases" or "transferases" (reaction 40). The substrates for these enzymes are the corresponding glucose-1-phosphates, compounds which contain glucose units already glycosidically bound to other sugar or aglycone residues, and finally, the sugar nucleotides. It is obvious in looking at these substrates that the source of energy for formation of di- and polysaccharides ultimately derives from ATP. Thus, the glucose-1-phosphates are formed either directly by the action of kinases or indirectly by kinases followed by mutases. As previously discussed, the sugar nucleotides also are derived from glucose-1-phosphate units. Finally, disaccharides (X = carbohydrate in Figure 6) also are derived either from the sugar nucleotides or the sugar phosphates. To reiterate, therefore, ATP ultimately provides the energy necessary for polysaccharide synthesis.

In reviewing the general reactions outlined in Figure 6, the glycosidases (reaction 38) might be considered briefly. Under certain conditions glycosidases transfer the glucose residue to acceptor molecules other than water. This type of reaction is similar to that of the transglycosidases; the present line of separation of the two types of enzymes depends upon the experimental conditions which are used, such as the relative concentrations of water and acceptor molecules, the ratio of transferring to hydrolytic activity, etc. It seems conceivable that a spectrum of enzymes of this sort exists rather than the two separate groups, and that under certain conditions within the cell, enzymes which are

presently considered to be glycosidases may actually possess transglycosidase activity.

*Enzymatic Synthesis of Disaccharides and Simple Glycosides.* While the monosaccharide units of most of the mucopolysaccharides are pyranosides of the  $\beta$ -glycosidic configuration, the naturally occurring D-glucose-1-phosphates and their corresponding nucleotide derivatives are generally of the  $\alpha$  configuration. A transfer reaction from the phosphate or the nucleoside diphosphate residues to yield disaccharides or polysaccharides therefore involves an inversion at C-1 of the glucose residue. Only a few inversions of this sort have been studied in detail:

(1) Maltose (containing the  $\alpha$ -glycopyranosidic bond) is converted to glucose and  $\beta$ -glucose-1-phosphate in the presence of inorganic phosphate and extracts from *Neisseria meningitidis* [25]. The reaction is reversible with an equilibrium constant of 4.4 in the direction of maltose synthesis.

(2) Cellobiose (containing the  $\beta$ -glucopyranosidic bond) is also cleaved by a specific phosphorylase and inorganic phosphate to glucose and  $\alpha$ -glucose-1-phosphate [82,83]. Here again, the equilibrium constant favors disaccharide formation.

(3) Glucuronide synthesis utilizing liver microsomal preparations requires UDP-glucuronic acid plus an acceptor. The glucuronide which is formed is of the  $\beta$ -configuration, whereas the substrate is presumably of the  $\alpha$  configuration. The acceptors in this case include phenolic, alcoholic, carboxylic acid, and amino compounds [84-86]. It may be of interest to note that the jaundice of the newborn appears to be due to inability to form bilirubin glucuronide. This is an enzymatic defect in the fetus and the newborn, and is apparently associated with a low-level of transferase and UDP-glucose dehydrogenase activities [87-91]; in the guinea pig, the enzymatic activities increase with age [87].

The transfer of a glucose residue from the corresponding phosphate or nucleoside diphosphate derivative to an acceptor molecule does not necessarily involve inversion of the glycosidic bond. Thus, extracts from plants catalyze the transfer of glucose from UDP-glucose to either fructose or fructose-6-phosphate, yielding sucrose or sucrose-phosphate [92,93]. The mechanism of synthesis of this important disaccharide has intrigued the biochemist for many years, and these studies have apparently resolved this question. A reaction analogous to sucrose syn-

thesis is the synthesis of trehalose-phosphate by an enzyme obtained from yeast [94]. Here the reaction involves the transfer of glucose from UDP-glucose to glucose-6-phosphate. Trehalose, like sucrose, contains an  $\alpha$ -glucosidic bond.

Another important disaccharide is lactose, which is a  $\beta$ -galactosyl-glucose. In this case, extracts obtained from bovine mammary tissue [95] yielded lactose-1-phosphate when incubated with UDP-glucose and glucose-1-phosphate, presumably through the intermediate formation of UDP-galactose from the UDP-glucose. While the intermediates for this synthesis have not been clearly established, an inversion around the C-1 of the galactose residue is suggested.

**Polysaccharide Synthesis.** Chitin, the simplest mucopolysaccharide, provided the first clear demonstration that sugar nucleotides are involved in polysaccharide synthesis [96]. Extracts obtained from *Neurospora crassa* converted UDP-AcGm to chitin in the presence of added chitodextrins. The chitodextrins were prepared by partial hydrolysis of chitin and presumably acted as "primers" or acceptor molecules for the N-AcGm residues transferred from the UDP-AcGm. In these experiments a net synthesis of the polysaccharide was observed. In a similar study, cellulose synthesis was noted with extracts of *Acetobacter xylinum* and  $C^{14}$ -labelled UDP-glucose plus cello-dextrins as "primers" [97]. In the case of cellulose synthesis, radioactivity was incorporated into the cellulose, but net synthesis was not reported. In the case of both chitin and cellulose, the  $\alpha$ -glucose nucleotides are converted to  $\beta$ -linked polysaccharides. Many questions remain unanswered; for example, both chitin and cellulose exist as fibrils, but the mechanism of fibril formation has not yet been clarified. Further, net cellulose synthesis has not yet been observed; nor has the mechanism of cellulose synthesis in plants been elucidated despite many attempts in this direction.

Another transferase reaction of potentially great importance is that which produces glycogen from UDP-glucose and either glycogen or soluble starch as "primer" [98]. In this case, net synthesis of glycogen was observed. Here there is no inversion of the glycosidic bond. It has been suggested\* that the transferase enzyme which yields glycogen acts in a synthetic capacity while glycogen phosphorylase acts in the degradation of glycogen. There are other possibilities to explain the presence of both the phosphorylase

\* Dr. L. F. Leloir, private communication.

and transferase activities in liver. The transferase enzyme may be the dominant enzyme in certain muscle preparations.

The synthesis of hyaluronic acid (HA) is even less well defined than that of the simpler polysaccharides already discussed. With labelled UDP-AcGm and UDP-GA and extracts of Rous sarcoma [99], a product was obtained containing radioactivity which was thought to be HA. However, relatively little of the  $C^{14}$  was incorporated, and significant losses of radioactivity were noted on purification of the HA. Extracts from Group A streptococci yielded more definitive results [100]. Again it should be noted that a  $\beta$ -polymer is formed from the  $\alpha$ -sugar nucleotides. It is difficult to visualize this synthesis as proceeding with a single enzyme since two substrates are required and must be linked in an alternating sequence in the polymer. A number of possibilities may be considered in this connection, such as the formation of nucleotide disaccharide, or the existence of two enzymes, one specific for the hexosamine residue, the other for the uronic acid residue. Detailed studies on the mechanism of synthesis of this polysaccharide should clarify these questions.

Enzymatic studies on the biosynthesis of only one other mucopolysaccharide have been reported—chondroitin sulfate. Preliminary reports indicated that the polysaccharide was formed when extracts of embryonic cartilage were incubated with ATP and inorganic sulfate [101, 102]. These surprising results suggest that all the carbon sources for the formation of the polysaccharide were present in the extracts. Extension of these findings should lead to an understanding of the mechanism of synthesis of one of the more complex connective tissue mucopolysaccharides. At present, there is no information as to the nature of the intermediates involved in the synthesis.

#### MISCELLANEOUS PROBLEMS

The information summarized in the foregoing pages outlines the extent of our information on the *in vitro* biosynthesis of some of the homopolysaccharides and the simpler heteropolysaccharides. As we proceed to the complex heteropolysaccharides, such as the bacterial and plant gum polysaccharides (which contain more than three types of monosaccharide units, phosphate, sulfate and the like), the problems become infinitely more complex. As long as a poly-



saccharide can be visualized as consisting of simple repeating units joined together like bricks in a brick wall, the mechanisms herein discussed may be operating. However, as soon as the concept of a simple repeating unit fails, the problems become as difficult to visualize as do protein and nucleic acid syntheses. It seems reasonable, therefore, to suggest that the more complex polysaccharides may be synthesized via some preformed template, as is presumed to be the case with protein, and does not proceed by simple polymerization steps.

While this review has considered only the enzymatic reactions involved in synthesis of mucopolysaccharides, numerous other problems await investigation in this field. Obviously, there is a good deal yet to be learned about the metabolism of the monosaccharide constituents. Only sparse information is available on the synthesis of the mucopolysaccharides *per se*. The metabolism of the glycoproteins and mucoproteins is essentially unknown. The effects of nutrition, hormones, and the like, on mucopolysaccharide metabolism are grossly evident, but the mechanisms are not understood. The "connective tissue diseases" apparently are biochemical diseases in the sense that normal metabolic patterns of connective tissue have changed, but there is no information as to the enzymatic disorders. For example, patients with Hurler's syndrome exhibit high concentrations of one of the mucopolysaccharides in liver and urine [103]; is this disease similar to a glycogen storage disease? If so, what is the enzymatic defect?

In reviewing mucopolysaccharide metabolism, at least a word should be said about mucopolysaccharide function. What are the physiological functions of the mucopolysaccharides apart from certain obvious ones such as the action of hyaluronic acid as a lubricant in the joints by virtue of its property of increasing the viscosity of joint fluid? Possibilities along these lines include maintenance of tissue structure, transport of metabolites to and from cells, resistance to mechanical stress, and water binding [104]. Other functions may be much more important. For example, many immunological phenomena appear to be associated with the mucoid substances; chemical explanations for these phenomena must be found. Finally, the relationship between proteins, particularly the fibrous proteins such as collagen and the mucopolysaccharides, is more often assumed than proved. What exactly is this relationship, and

how is it affected by such factors as nutritional states, hormones, and the like?

*Acknowledgment:* The editorial assistance of Mrs. Miriam Butsch is gratefully acknowledged.

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# Clinical Studies

## The Concept of Functional Coarctation of Large Blood Vessels\*

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WHILE it is agreed that not all narrowed segments of blood vessels require surgical correction, there is at present no accepted way to quantitate the degree of great vessel stenosis (coarctation). The recent reports of asymptomatic single or multiple postvalvular pulmonary stenosis suggest that anatomically demonstrable stenosis might be present without necessarily a functional impairment in flow. Since the main function of the large arteries is to provide blood to the systemic or pulmonary circulation at a pressure head within a wide range of normal limits, it is not likely that the mean pressure alone distal to the lesion could be considered a sensitive index of functional impairment. The production of a definite pressure gradient at a local site, however, might allow embarrassment of vascular supply distal to the site of the pressure drop under certain conditions requiring high flow rates.

A second characteristic of normal blood flow in arteries is its pulsatile nature. Again, normal variations are so wide that a single local pulse pressure value is not likely to reflect minor degrees of interference by vascular stenosis. Because of the distortion of pulse wave contour by various factors at the periphery, even the simultaneous recording of pressures at multiple sites might not reflect a more proximal abnormality.

A third characteristic of normal flow through the arterial bed is the shape of the pulse wave. Specifically, mean pressure and pulse pressure may be normal or within the normal range, but the rate of initial pressure rise, the slope, measured in mm. Hg/second, may be altered. In normal subjects at rest, the values for slope fall

into a rather discrete range [1]. In vascular stenosis, the slope distal to a narrowed segment may be greatly reduced with the values of pulse pressure, mean pressure and flow still within normal range. The demonstration that the pressure receptors of the carotid sinus are sensitive to the rate as well as to the magnitude of pressure changes [2] suggests that this parameter might be of physiological importance.

With the foregoing possible theoretical abnormalities in mind, and in a search for a simple and precise diagnostic technic, the following method was devised to describe the actual parameters affected by anatomical stenosis of the great vessels in man. It is based on the simultaneous recording of multiple "central" pulses by means of appropriate arterial catheters used for pulse analysis by Peterson et al. [3], Warner et al. [4] and many others. Its clinical usefulness in certain valvular defects has been described [1].

### METHODS AND MATERIAL

Four human subjects with clinical diagnosis of coarctation of the aorta were studied. Two arterial catheters (1 mm. external diameter) were made from stiff plastic tubing† and standard fittings, and were passed through a No. 18, thin-walled needle into a brachial and a femoral artery. Simultaneous pressure pulses were obtained from segments proximal and distal to the coarctation (as close to the defect as possible), using a Statham strain-gauge manometer system [1].

The slope was calculated as the initial rate of rise of pressure in mm. Hg/second. The mean pressures were obtained by planimetry.

‡ Obtained from Albert E. Afford, Barrington, New Jersey.

\* From the Department of Medicine, University of Utah College of Medicine, Salt Lake City, Utah. Supported in part by a grant from the Utah Heart Association, the U. S. Public Health Service (National Heart Institute), and the Wyeth Laboratories. This work was carried out during the tenure of an American Heart Association Fellowship.

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TABLE I  
PRESSURE DATA IN PATIENTS WITH AORTIC COARCTATION

Case No.	Sex and Age (yr.)	State	Heart Rate	Pressure (mm. Hg)				Slope (mm. Hg/sec.)		Distance (cm.) of Defect from Femoral Artery
				Proximal		Distal		P	D	
				S/D	Mean	S/D	Mean			
I	M, 31 34	Preoperative	65	189/96	130	123/88	107	670	144	52
		Postoperative (3 yr.)	70	132/75	98	132/74	97	580	560	53
II	M, 26 28	Preoperative	80	144/98	117	121/96	105	955	144	59
		Postoperative (2 yr.)	84	146/81	106	133/85	102	740	364	59
III	M, 7	Priscoline*	96	147/70	100	115/73	97	1072	604	..
		Preoperative	90	132/72	103	92/72	82	750	104	35
IV	M, 10	Preoperative	70	120/65	97	113/65	92	500	460	30

NOTE: P = Proximal from coarctation.

D = Distal from coarctation.

S/D = Systolic and diastolic.

Slope = Rate of rise of ascending limb of pressure curve.

\* Measurements made twenty minutes after 50 mg. of Priscoline was administered intravenously.

#### CASE REPORTS

**CASE I.** E. C., a man aged thirty-one, was admitted to the Salt Lake Veterans Administration Hospital on May 31, 1955, for investigation of hypertension refractory to treatment. Hypertension had been noted on entering the Navy at the age of nineteen. At the age of twenty-two the patient was hospitalized for investigation of elevated blood pressure. No symptoms except occasional headaches were recorded. The elevated blood pressure did not respond to the administration of reserpine and Apresoline.<sup>®</sup> Physical examination showed a blood pressure of 180-200/80 mm. Hg in the upper extremities; it was not obtainable in the lower extremities. The arterial pulsations were bounding in the upper portions and pedal regions of the body but barely palpable in the femoral region and absent in the popliteal region. The fundi were normal. An apical systolic cardiac murmur of moderate intensity was present. There was a palpable thrill over the right internal mammary artery and over the upper intercostal arteries in the back. The heart was not enlarged on x-ray examination, but rib-notching was seen. The electrocardiogram was interpreted as being normal. At surgery, resection of a coarcted segment at the level indicated by catheter exploration (see text) was accomplished, and despite repeated separation of the anastomosed vessel a good postoperative result was obtained.

**CASE II.** J. B., a man aged twenty-five, was admitted to the Salt Lake Veterans Administration Hos-

pital on May 15, 1956, with the complaint of headaches of one year's duration. Hypertension had been noted at the age of twelve. Two weeks prior to admission a chest roentgenogram, which showed rib-notching, and inability to obtain blood pressure readings in the lower extremities suggested the diagnosis of coarctation of the aorta. The blood pressure in the arms was 190/110 mm. Hg; it was unobtainable in the lower extremities. The femoral pulses were weak; pedal pulses were absent. The pulses in the upper extremities were forceful. A basilar systolic murmur was present. The electrocardiogram was within normal limits. X-ray examination of the chest showed, in addition to rib-notching, a small aortic knob and dilatation of the upper descending aorta. Surgical exploration revealed the coarcted segment as indicated by catheter studies but there was also a length of hypoplastic vessel which was not amenable to resection and replacement. Although there was postoperative lessening of hypertension, the pressure in the upper extremities did not return to normal and postoperative studies twenty months later showed a functional defect remaining. (Table 1.)

**CASE III.** B. S., a boy aged seven, was admitted to the Salt Lake General Hospital on November 17, 1957, for investigation of a cardiac murmur present since the age of three. The patient developed normally and was asymptomatic and non-cyanotic. The blood pressure was 120/80 mm. Hg in the upper extremities; and 100/60 mm. Hg in the lower extremities with diminished femoral pulsations. A systolic murmur was

heard over the pulmonic area and the left intrascapular area. X-ray examination of the chest did not show any abnormalities and the electrocardiogram was within normal limits. Catheterization of the right side of the heart showed normal values for pressure and blood saturation in the various chambers and a dye dilution curve was normal. The results of arterial catheterization are summarized in Table 1.

CASE IV. G. H., a white boy aged seven, was seen at the Salt Lake General Hospital on December 8, 1955, for evaluation of congenital heart disease suggested by the presence of a cardiac murmur first heard at the age of five. Birth, growth and development were normal. There was no exercise intolerance, and no cyanosis. Physical examination showed a blood pressure, taken sequentially, of 124/70, 128/70 mm. Hg in the left arm; 130/68 and 124/66 mm. Hg in the right arm; 120/90 and 118/86 mm. Hg in the right leg; 118/88 and 120/90 mm. Hg in the left leg. A loud, harsh, high-pitched, grade 2 systolic murmur was heard in the aortic area. All pulses were palpable. Cardiac fluoroscopy showed a normal-sized heart with no evidence of structural alteration of the aorta or thoracic collateral circulation. Arterial dye dilution curves following venous injection were normal in contour and simultaneous brachial and femoral arterial pressures were obtained by direct needle puncture. Angiocardiogram on this admission showed the findings indicated in Figure 2. The child was again seen at ten years of age when arterial studies were repeated. (Table 1.) The patient has continued to develop normally, and is presently asymptomatic. The electrocardiogram has been within normal limits.

#### RESULTS

The results are summarized in Table 1. In three patients a drop in both systolic and mean pressures and a decrease in the slope were noted in the aortic segment distal to the coarctation, indicating a physiologically significant defect. (Fig. 1A.) The possible artefacts due to local turbulent flow were not apparent in the distal pressure pulse. Figure 1B shows two proximal pulses in the same patient, after the distal catheter was passed into the proximal segment through the defect; the increase in the value of the proximal slope as compared to Fig. 1A is perhaps due to momentary changes in flow. Further, the identity of the two pressure pulses indicates that the direction of the catheter orifice (upstream or downstream) did not measurably affect the pressure values. Passing the catheter through the defect and thereby accurately localizing the site of constriction was possible in another subject (J. B.). In still another patient, the

catheter could be wedged from below into the coarcted segment.

The postoperative findings in Case I, (E. C.) three years after surgery, are within normal limits. (Fig. 1C.) Due to technical difficulties, the surgeon was not satisfied with the repair in Case II (J. B.). This was reflected in the study made two years postoperatively. (Table 1.) The value for slope in the distal segment, even though higher than the preoperative value, was still less than the simultaneously measured slope in the proximal segment. A small systolic gradient was also present. When 50 mg. of Priscoline® was administered to increase cardiac output, both slopes (proximal and distal) increased; however, the differences between the two slightly increased. This shows clearly that the slope in the individual pulse, proximal or distal, can change with instantaneous changes in flow; however the difference between the two slopes persists. In Case II (J. B.) the distal slope by itself was still within normal limits (normal range of slope 538 mm. Hg/second  $\pm \sigma$  86  $\pm \sigma_m$  24) [7]. The dependence of the degree of pressure gradients on flow is expected and follows the same general rules that apply to pressure gradients observed across the valve in mitral stenosis.

It should be made clear that for measurement of slope, peripheral pulses cannot be used because of the distortion that a pulse wave undergoes as it traverses to the periphery. The proximal and distal pulses must be obtained as close to the defect as possible. The anatomical site of the coarcted area can then be estimated by the length of the catheter needed to reach the defect.

The method was helpful in ruling out physiologically significant coarctation in Case IV (G. H.).\* In spite of obvious vascular obstruction seen by angiocardiography (Fig. 2), the patient had neither a significant decrease in the distal slope nor a systolic pressure gradient between the proximal and distal pressure pulses. (Table 1.)

Thus, it seems that a decrease in the value of the distal slope as compared to rate of rise of the proximal pulse at a given instant is a constant and sensitive index of the functional degree of stenosis of a large artery. This was also observed in cases of coarctation of a branch of the pulmonary artery previously reported [5], wherein the slope of the pressure pulse decreased abruptly as

\* This patient was seen by courtesy of Dr. L. George Veasy, Salt Lake City, Utah.



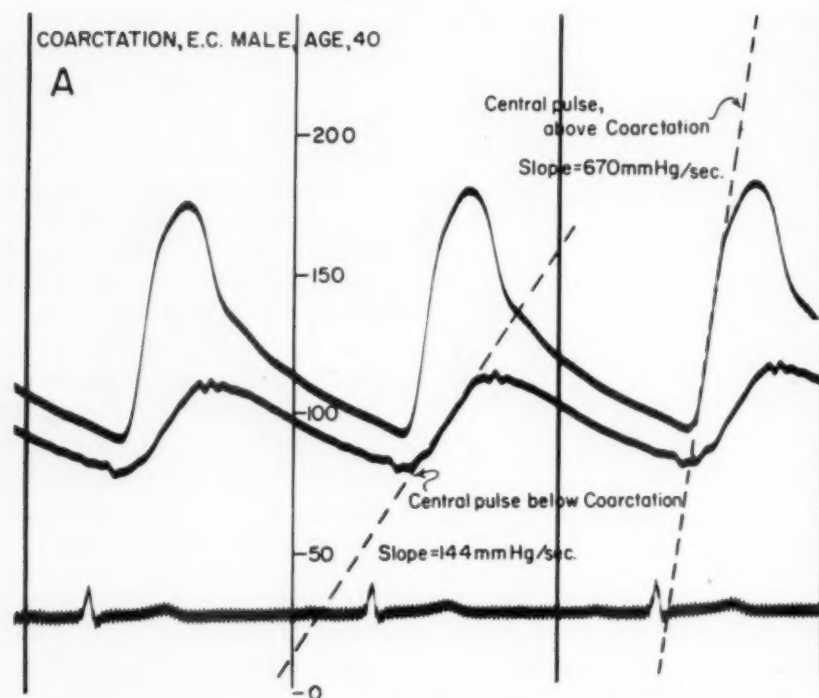


FIG. 1A. "Central" pulses, obtained by retrograde arterial catheterization, proximal and distal to coarctation of the aorta. Note the decrease in slope and systolic pressure in the distal pulse. (Central pulse below the coarctation.)

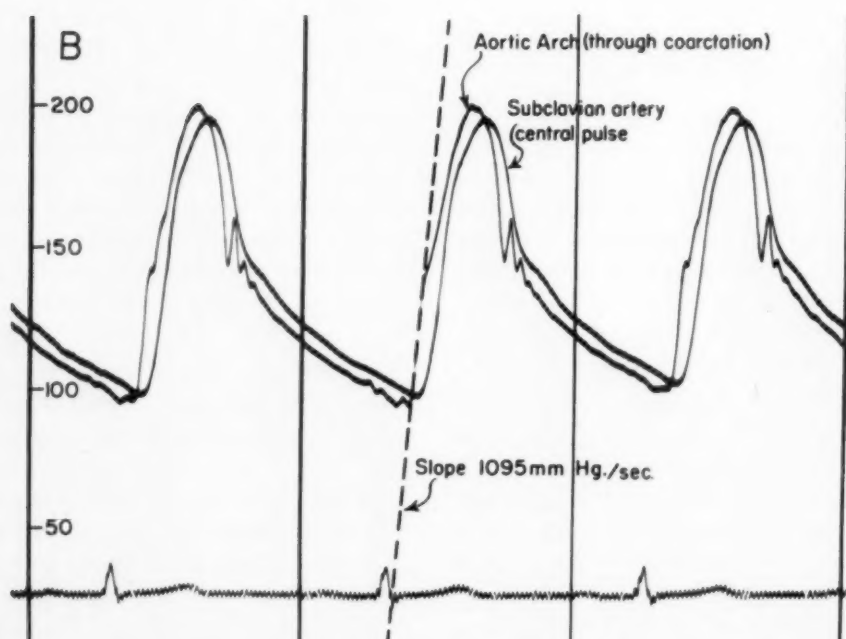


FIG. 1B. The distal catheter was passed through the defect into the proximal segment.

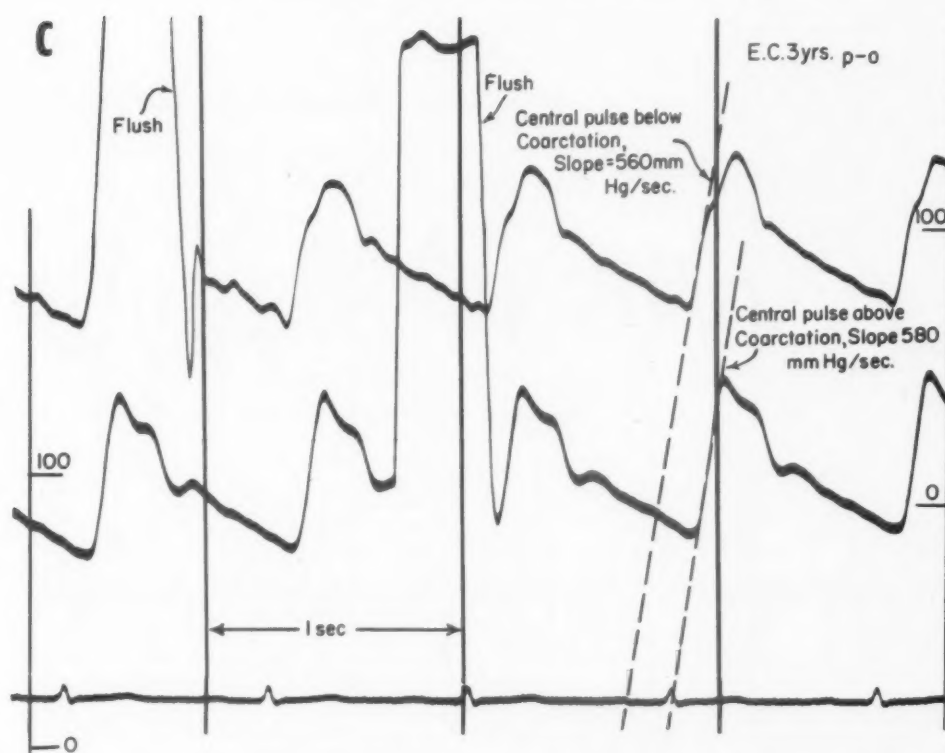


FIG. 1C. Study as in Figure 1A, three years postoperatively.

the defect was traversed by a cardiac catheter. (Fig. 3.)

It is likely that the anatomic defect alone could account for most of the pressure phenomena of the coarctation [6]. These could be reproduced in cats by placing an acute constriction around the descending aorta, and representative results are shown in Table II. A hydraulic model (Fig. 4) in which a constriction (RC) was produced in a distensible rubber tubing could also reproduce the characteristic pressure pulses found in coarctation when water was pumped through the tubing with a rubber bulb with one way valves. (Control pressure pulses were obtained by removing the constriction.) The relationship between the proximal and distal diastolic pressures in the presence of a coarctation (RC) could be altered in the model. It was possible to obtain a distal diastolic pressure higher than the proximal diastolic pressure by increasing resistance  $r_L$  and decreasing  $r_U$ . (Table II.)

An electrical analogue of the coarctation of large blood vessels found in man can also be constructed. (Fig. 5A.) The capacitances (C) represent the distensibility of the vessel and a variable resistance (R) represents the coarctation. A normal arterial pulse is fed in the circuit as voltage input ( $e_{in}$ ) and a coarcted pulse could be obtained as voltage output ( $e_{out}$ ). The load

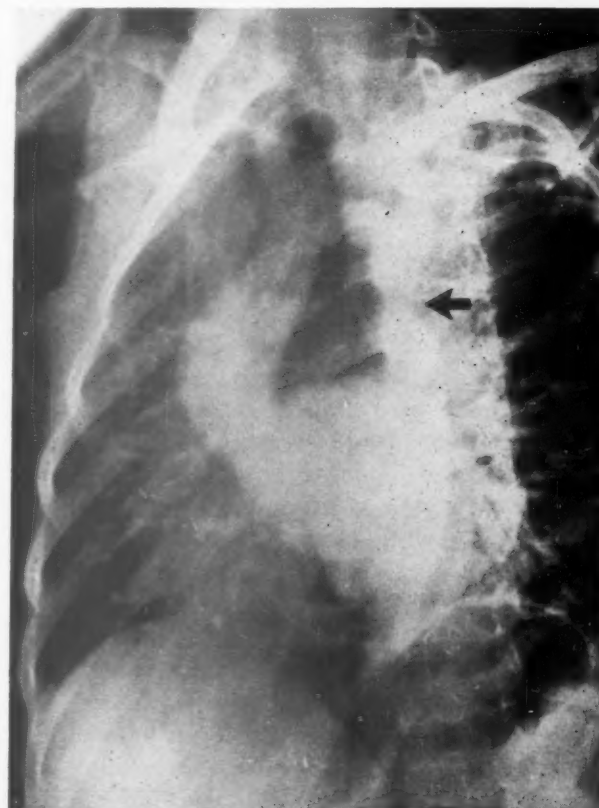


FIG. 2. Angiogram, showing anatomical narrowing of the aorta (arrow) in Case IV (G. H.). In spite of the clearly visible constriction, no significant pressure changes were noted. (Table I.)

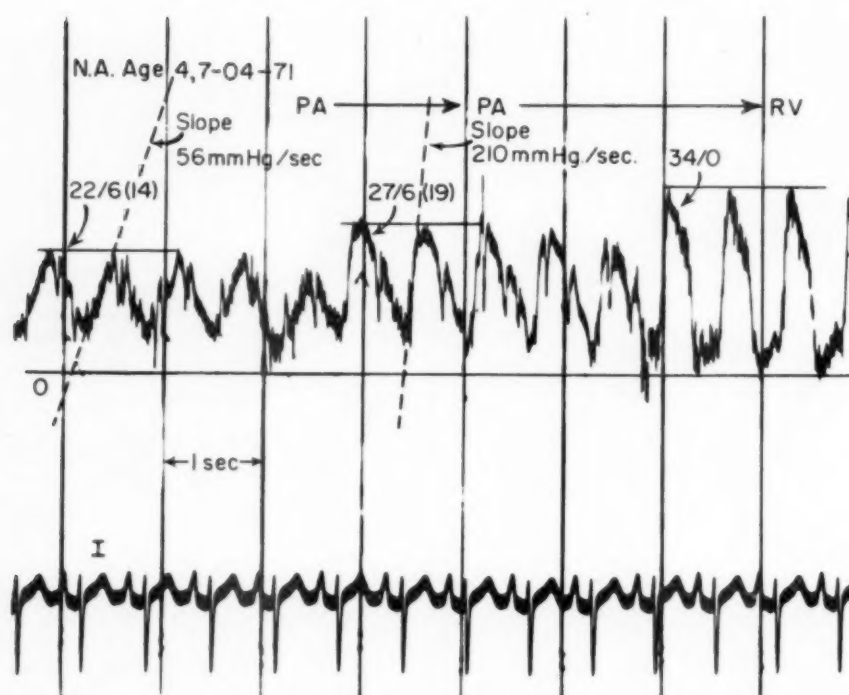


FIG. 3. Postvalvular stenosis of pulmonary artery, demonstrated by an abrupt change in slope, as well as pressure changes, as the catheter was withdrawn from a distal branch of the pulmonary artery. Case of Williams, Lange and Hecht [5].

TABLE II  
SOME EXAMPLES OF EXPERIMENTAL PRODUCTION OF COARCTATION

Coarctation Produced In:	State	Heart Rate	Pressure (mm. Hg)				Slope (mm. Hg/sec.)		Remarks
			Proximal		Distal		P	D	
			S/D	Mean	S/D	Mean			
Cat. ....	Control test	190	80/53	62	75/55	62	711	753	Mechanical obstruction, descending aorta
		180	75/52	60	53/45	47	685	293	
Hydraulic model: No. 1. ....	Control test	17	161/38	93	159/33	90	150	152	$r_U$ high; $r_L$ low (Fig. 4)
		20	225/42	136	122/31	80	325	93	
No. 2. ....	Control test	27	238/101	172	238/101	172	264	270	
		25	236/70	146	162/117	142	212	56	
									$r_U$ low; $r_L$ high (Fig. 4)

NOTE: P = Proximal from coarctation.  
D = Distal from coarctation.  
S/D = Systolic and diastolic.  
Slope = Rate of rise of ascending limb of pressure curve.  
Test = Coarctation induced.



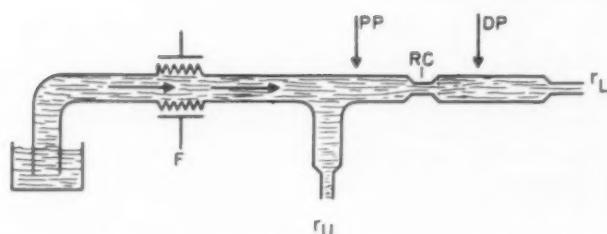


FIG. 4. Hydraulic model to simulate coarctation of the aorta (see text). F = pump with one way valves. RC = constriction (used to produce coarctation). PP = proximal pulse recording site. DP = distal pulse recording site.  $r_U$  = resistance proximal to the constriction.  $r_L$  = resistance distal to the constriction.

resistance  $r_L$  is the resistance of the galvanometer which measures  $e_{out}$ . (At this frequency the galvanometer offers primarily ohmic resistance.) By changing the values of  $R$  various degrees of coarctation could be simulated. (Figs. 5C and 5D.) With  $R = 0$  the pulse fed in could be reproduced faithfully, representing a control. (Fig. 5B.)

#### COMMENTS

The clinical observations and the analogue experiments seem fairly consistent. The use of two retrograde small arterial catheters is simple and does not require elaborate equipment. The diagnosis of coarctation of the aorta can be made with precision both as regards the degree of constriction as well as the location. Using simultaneous flow data one is tempted to calculate the internal diameter of the lesion but this may lead, of course, to serious errors because of the proximal collateral run off. The demonstration in Case III of the presence of a constriction by angiocardiology, which was physiologically of little consequence, indicates the usefulness of the proposed technic in further evaluating aortic constrictions which may occur more commonly than suspected. Surgery is still formidable and direct pressure measurements close to the site of the constriction may aid in the presurgical evaluation of these patients as well as providing a simple check on the surgical results obtained. (Table I.) Robicsek [10], using a single retrograde catheter, obtained arterial "pullthrough" pressures in several cases of coarctation.

All clinical and experimental observations demonstrate a lowering of the systolic peak pressures distal to the lesion with the diastolic pressure only slightly changed. This well known phenomenon points to the role of the distant peripheral resistance ( $r_L$  of Fig. 4) which is likely

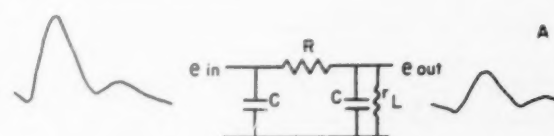
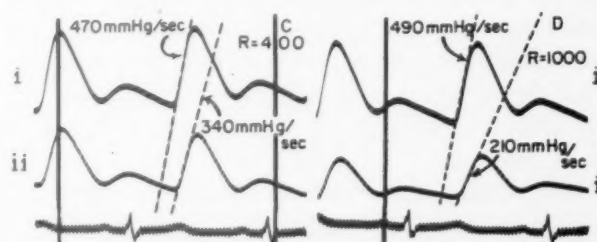


FIG. 5A. Electrical analogue of the coarctation of a large blood vessel (see text).  $R$  = variable resistance, representing coarctation.  $C$  = capacitance representing distensibility of the blood vessel.  $r_L$  = resistance offered by the galvanometer.  $e_{in}$  and  $e_{out}$  = voltage input and output, respectively.



FIG. 5B. I = radial artery, pressure pulse directly recorded serving as a control. II =  $e_{out}$  recorded after radial pulse (as in I) was fed in as  $e_{in}$  when  $R = 0$ .



FIGS. 5C and D. C, I = radial artery pulse as in B. II = as in B II with  $R = 400$  ohms. D, I = radial artery pulse as in B. II = as in B II with  $R = 1000$  ohms.

to be increased under such circumstances thereby raising the mean pressure head available for distal flow. It was of interest that increasing distal peripheral resistance in the hydraulic model raised the diastolic pressure below the coarctation appreciably above that obtained proximately (Table II) which tends to indicate that the peripheral circulation is supported by the collateral flow into a more or less constricted vascular bed.

The measurement of the slopes of the curves obtained in the immediate proximity of the constriction appears as another very useful parameter that is dependent on flow but essentially independent of the actual pressure values, an observation that supports the same findings made by the use of the central pulse in acquired aortic valvular disease [7]. It is a sensitive indicator to which might be added the known phase shift of the distal curve which in coarctation generally results in a later onset of the pressure

rise below the coarctation. The records of Figure 1 and of those obtained through the electric analogue (Fig. 5) demonstrate this particular diagnostic feature.

It is realized that the situation in coarctation of the aorta is more complicated but the basic mechanism here presented may play an important role. It has been suggested that actual flow through the coarctation may be slight or virtually absent and that the segments are only connected by the collateral channels [7]. If this were the case, the collateral channels could constitute a resistance between the two aortic segments and would cause similar hemodynamic phenomena provided their effective resistance was high. It seems that at least in patients in whom the catheter tip can be passed through the defect, or in whom the transmission of the pressure pulse between the two segments is virtually instantaneous (Fig. 1A), actual flow occurs through the coarctation. The pathological finding of a very narrow lumen in a section of the coarcted area does not necessarily rule out a patent channel in the lining with a high pressure head proximal to the defect [8,9].

#### SUMMARY

1. Passing two arterial catheters into the central portion of the arterial circulation, above and below a suspected area of constriction, allows a simple and precise localization of coarctation.

2. Analysis of pressure differences across the defect includes changes in systolic and in mean pressures, and in measurements of the slope of the curves. The latter is considered a particularly sensitive index of functionally significant obstruction.

3. These physiologic parameters may be normal in the face of an anatomically demonstrable narrowing of the aortic channel. Under these circumstances surgical repair seems unnecessary.

4. The pressure changes of coarctation can be simulated by appropriate hydraulic and electric analogues which emphasize that alterations in flow through the narrowed channel alone may be sufficient to reproduce many of the clinical findings.

*Acknowledgments:* The authors are particularly indebted to Mr. Arthur Ruby for his valuable assistance in the experimental part of the study.

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# Cortisone-Induced Polyuria Following Hypophysectomy\*

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THE development of polyuria following hypophysectomy in patients treated with cortisone has been observed on numerous occasions. Ikkos, Luft and Olivecrona [1] were among the first to show that the persistence of polyuria after the third postoperative week was dependent upon cortisone administration. On the basis of observations on their patients they suggested that polyuria was due primarily to the need "for a larger amount of water for the excretion of a given solute load" and secondly to an increase in the urinary solute load due either to an increased dietary intake or to the metabolic effect of the hormone. The first of these explanations involves the assumption that cortisone diminishes the ability of the renal tubules to reabsorb solute-free water, and produces or aggravates diabetes insipidus.

In 1952, Leaf, and his associates [2] carried out extensive investigations on a patient suffering from diabetes insipidus whose thirst and polyuria disappeared following the development of insufficiency of the anterior pituitary. In this patient these workers showed that the amelioration of the polyuria was merely the result of a reduced solute excretion due to diminution in appetite and that, as long as the patient ingested a constant diet, the administration of cortisone did not alter water excretion. This conclusion was consistent with the older observations of Winter, Ingram and Eaton [3] and of Beaser [4] who had shown that the magnitude of the polyuria in experimental diabetes insipidus was directly proportional to the excreted solute load.

Stribling and Spurr [5], in a brief report on one patient suffering from insufficiency of the anterior and posterior pituitary in whom polyuria developed while he was receiving a constant diet and 100 mg. cortisone, thought that the polyuria was the result of a decreased tubular reabsorption of water but suggested that, in addition, the

thirst mechanism was stimulated by an increase in sodium retention induced by the hormone. A similar explanation was invoked by Skillern, Corcoran and Scherbel [6] for the occurrence of polyuria in one patient suffering from coincident diabetes insipidus and Addison's disease. This idea assumes that sodium reabsorption induced by cortisone is unaccompanied by an increase in water reabsorption, a suggestion which is not supported by Stribling and Spurr's own observations that the osmolality† of their patient's urine after twelve hours water deprivation was not reduced by cortisone administration.

There have been other reports of the occurrence of polyuria following hypophysectomy in patients receiving cortisone [7,8]; in these it is generally assumed that the syndrome is merely a manifestation of diabetes insipidus and the authors did not enquire further into its mechanism. There is thus no unanimity of opinion as to the mechanism by which cortisone induces the polyuria, nor is it known whether or not the cases represent a single clinical entity.

We have investigated two patients in whom, following hypophysectomy, severe persistent polyuria and polydipsia developed as a result of the administration of cortisone. Both patients have been studied in order to elucidate the following points: (1) the relationship between the occurrence of polyuria and the administration of cortisone, independent of any alteration in the load of urinary solute, (2) the relationship between the occurrence of any cortisone-induced polyuria and certain parameters of renal function specifically related to the power to conserve water, namely: (a) the osmolal concentration of the urine following twenty-two hours of food and

† The term "osmolality" is used throughout in preference to "osmolarity" as expressing the particulate concentration per unit volume of plasma or urine, rather than per unit volume of plasma water or urinary water.

\* From the Departments of Clinical Chemistry and Therapeutics, University of Edinburgh, Scotland.



water deprivation with and without the administration of Pitressin®; (b) the capacity of the distal concentrating mechanism of the kidney to reabsorb water free of solute under conditions of hydropenia and osmotic diuresis with and without the administration of Pitressin.

In this way it was hoped to define more clearly the mechanism involved in the production of the syndrome, and to determine particularly whether the polyuria is due to a direct or indirect effect of the hormone on the capacity of the distal concentrating mechanism of the kidney to reabsorb solute-free water\* or to a primary polydipsia with consequent polyuria.

#### CASE REPORTS

**CASE I.** E. B., a thirty-nine year old woman, underwent hypophysectomy for metastatic carcinoma of the breast in November 1956. She was given 100 mg. cortisone acetate on the day of operation and became aware of a troublesome thirst on the first postoperative day. Administration of cortisone was continued, and thirst and polyuria persisted after her discharge from hospital despite the reduction of her daily dose of cortisone to 12.5 mg. In February 1957, she was admitted to a metabolic ward for investigation.

On examination she was a moderately obese woman with a pale, dry, cold, finely wrinkled skin. She complained of undue sensitivity to cold and showed mental and physical retardation. The blood pressure was 120/85 mm. Hg and the urine, which was sterile on culture, contained no protein. Microscopic examination of the urinary sediment revealed no abnormality. The serum electrolytes estimated on admission were as follows: Na 141.0 mEq./L., K 4.4 mEq./L., Cl 92.6 mEq./L., HCO<sub>3</sub> 31.7 mEq./L. The blood urea nitrogen was 12 mg./100 ml. and the serum cholesterol 354 mg./100 ml. Radioactive iodine studies confirmed the diagnosis of hypothyroidism, 91.3 per cent of a tracer dose of I<sup>131</sup> being excreted in forty-eight hours, while the "T index" was only 1.3 (preoperative values: zero to forty-eight hour excretion 32 per cent, T 6.8). Urinary 17-ketosteroid excretion was determined by the method advised by the Medical Research Council Committee [9] and values of 2.7, 3.0 and 4.0 mg./twenty-four hours were obtained on three successive days (normal range 4 to 14 mg./twenty-four hours). Urinary gonadotrophins [10] were less than 3 human menopausal gonadotrophin (HMG) units/twenty-four hours. This patient was obviously suffering from thyroid and adrenocortical insufficiency secondary to hypopituitarism.

\* "Solute-free water" is the conventional, if tautological expression used to describe the amount of water reabsorbed or excreted by the kidney in excess of the amount needed to maintain isotonicity between the urine and the plasma.

**CASE II.** M. M., a forty-seven year old woman, underwent hypophysectomy for metastatic carcinoma of the breast in October 1957. At the time of operation radiogold was inserted into the sella turcica. Cortisone acetate was given on the day of operation in a daily dose of 200 mg. and the dose was thereafter gradually reduced until in January 1958 she was receiving 37.5 mg./day. Immediately after hypophysectomy, polyuria and polydipsia developed. These symptoms persisted despite the reduction in cortisone dosage and were sufficiently severe to necessitate her re-admission to the hospital in January 1958 for investigation in a metabolic ward.

On examination she was a pale, thin, tired looking woman. The blood pressure was 120/70 mm. Hg. Serum electrolytes were: Na 130.4 mEq./L., K 4.5 mEq./L., Cl 92.0 mEq./L., HCO<sub>3</sub> 26.0 mEq./L. The blood urea nitrogen was 13 mg./100 ml. The urine contained no protein and was sterile on culture. Examination of a freshly passed specimen after centrifugation revealed a normal sediment. Urinary gonadotrophins were 7 and 5 HMG units/twenty-four hours following operation, having been 66 HMG units/twenty-four hours on one preoperative day. The twenty-four-hour urinary excretion of 17-hydroxysteroids determined by the method of Forsham *et al.* [11] proved to be so low as to be unmeasurable. Urinary 17-ketosteroids estimated by the method of Moxham and Nabarro [12] were 4.2 and 5.0 mg./twenty-four hours on two successive days (normal range 7 to 20 mg./twenty-four hours). Radioactive iodine studies showed no evidence of impairment of thyroid function. During periods when the patient was deprived of cortisone she became unwell and complained of lassitude, anorexia and nausea. On these occasions a mild pyrexia developed. The patient suffered from moderately severe adrenocortical insufficiency as a consequence of hypopituitarism.

#### METHOD OF INVESTIGATION

Throughout the entire period of study both subjects received a diet containing constant amounts of protein, fat, carbohydrate, sodium and potassium. The sodium content of the diet was 20 mEq./day and this was supplemented by 34 mEq. of sodium as oral sodium chloride. An unrestricted intake of water was permitted.

The following observations were made on both patients while they were receiving either cortisone acetate tablets or dummy tablets orally, with or without the administration of pitressin tannate intramuscularly or Pitressin (Parke, Davis) intravenously: (1) The volume of the twenty-four-hour urinary output and its osmolality were determined over periods of five to seven days while the patients received either cortisone or dummy tablets with or without the administration of pitressin tannate in oil by intramuscular injection. (2) Measurements were made at suitable

intervals of the osmolality of a specimen of urine excreted after twenty-two hours of food and water deprivation. The observations were repeated following the administration of 20 units of pitressin tannate given at the beginning of the period of hydropenia. (3) On these occasions, following the periods of hydropenia, osmotic diuresis was induced by the infusion of a 15 per cent solution of mannitol and measurements were made of the volume of solute-free water reabsorbed by the renal tubules per minute.

A priming dose of a 15 per cent solution of mannitol calculated, on the basis of body weight, to raise the osmolality of the extracellular fluid by about 20 mOsm/L., was administered intravenously, the injection taking a period of ten minutes. Thereafter the concentration of mannitol in the extracellular fluid was maintained by the continuous intravenous infusion of a similar solution at a rate of 4 to 10 ml./minute. Twenty minutes were allowed for equilibration after which urine was collected during several short periods of ten to fifteen minutes duration. Thereafter, depending on the rate of urine flow, the infusion was either stopped for a period or a second priming dose of mannitol was given and its extracellular concentration was sustained by continuing the infusion at an appropriate rate. After a suitable period of equilibration additional collections of urine were obtained. At this point, in Case II (M. M.), a priming dose of 200 milliunits of Pitressin (Parke, Davis) was given intravenously in four minutes and this was followed by the infusion of 150 milliunits of Pitressin per hour. This was achieved by the addition of a suitable amount of Pitressin to the sustaining infusion of mannitol which was then continued for an additional sixty minutes when four more collections of urine were made. In Case I (E. B.), intravenous Pitressin was not employed but separate determinations of the reabsorption of solute-free water were made after the administration of 20 pressor units of pitressin tannate in oil given daily for two days immediately prior to the period of hydropenia. The details of these observations are given in Tables I and II.

Urine was obtained by means of an indwelling multieyed catheter, air insufflation and suprapubic compression being employed to ensure adequate emptying of the bladder. Blood was withdrawn from an antecubital vein before the initial mannitol injection was given and further samples of venous blood were taken for each series of urine collections.

**Chemical Methods.** The osmolality of the urine was determined by measurement of the freezing point depression using a thermistor osmometer. Plasma osmolality was calculated from the formula (Brodsky [73]):

$$\text{osmolality} = 2[\text{Na}^+] + 2[\text{K}^+] + [\text{urea}] + 0.925[\text{mannitol}] - 8$$

Mannitol was determined chemically by the method of Todd et al. [14]. The glomerular filtration

rate was determined by the measurement of the clearance of endogenous creatinine during each collection period. Serum and urinary creatinine were measured by the modification of Haugen's method reported by Owen and co-workers [15]. Sodium and potassium in serum and urine were estimated by the Eel direct-reading flame photometer. Urea in the blood and urine was estimated by the manometric method of Peters and Van Slyke [16]. Serum and urinary chlorides were estimated by the method of Van Slyke [17].

**Calculation of the Volume of Solute-free Water Reabsorbed per Minute ( $T_{H_2O}^C$ ).** During osmotic diuresis the amount of solute-free water reabsorbed by the renal tubules is calculated by the difference between the rate of urine flow,  $V$ , and the osmolal clearance,  $UV/P$ , in which  $U$  and  $P$  represent the osmolal concentration of urine and plasma respectively [18]:

$$T_{H_2O}^C = \frac{UOsm \times V}{POsm} - V$$

The value of  $T_{H_2O}^C$  is positive when the kidney is concentrating urine above the osmolality of plasma and is negative when urine is less concentrated than plasma. When the urine is isosmotic with plasma the value of  $T_{H_2O}^C$  becomes zero.

## RESULTS

**The Occurrence of Polyuria with the Administration of Cortisone Acetate.** Figures 1 and 2 show the values of the twenty-four-hour urine volumes and their osmolalities in both patients during the administration of dummy tablets and tablets of cortisone acetate with and without the administration of pitressin tannate. While receiving dummy tablets the twenty-four-hour urinary volume of E. B. ranged between 3.3 and 4.4 L. It showed some increase following the administration of 25 mg. cortisone acetate per day and reached levels of more than 7 L./day when 300 mg. were given. The urine output of M. M. prior to the administration of cortisone was more nearly normal, ranging between 1.4 and 2.5 L. This volume also rose to about 7 L./day following the administration of a daily dose of 300 mg. cortisone. The injection of pitressin tannate in oil produced a fall in the twenty-four-hour urinary volume of both patients irrespective of whether cortisone was being given or not. The urinary volumes were reduced by this treatment to between 500 and 2,500 ml./twenty-four hours. The polyuria induced by cortisone was accompanied by intense thirst and a corresponding polydipsia but not by any significant change in weight. The

TABLE I  
PATIENT E. B.

Time (min.)	V (ml./min.)	POsm (mOsm./L.)	UOsm (mOsm./L.)	Osmolal Clearance (ml./min.)	T <sub>H<sub>2</sub>O</sub> <sup>c</sup> (ml./min.)	Creatinine Clearance (ml./min.)
<i>2/19/57—23 Hours Hydropenia</i>						
0	300 ml. intravenous 15% mannitol, infusion 15% mannitol 4 ml./minute					
30	Empty bladder	...	...	...	...	...
38	5.9	...	276	5.4	-0.5	96
45	6.3	302	255	5.3	-1.0	90
52	6.0	...	268	5.3	-0.7	84
58	150 ml. intravenous 15% mannitol, infusion 15% mannitol 6 ml./minute					
72	Empty bladder	...	...	...	...	...
79	8.9	...	298	8.6	-0.3	86
85	10.1	...	316	10.5	+0.4	91
91	8.3	302	318	8.8	+0.5	91
97	9.0	...	324	9.7	+0.7	111
				Average.....	-0.1	93
<i>3/1/57—23 Hours Hydropenia—25 mg. Cortisone Acetate/Day for 7 Days</i>						
0	200 ml. intravenous 15% mannitol, infusion 15% mannitol 8 ml./minute					
28	Empty bladder	...	...	...	...	...
40	7.7	...	352	9.4	+1.7	91
53	7.4	290	360	9.2	+1.8	85
62	8.7	...	348	10.5	+1.8	94
65	150 ml. intravenous 15% mannitol, infusion 15% mannitol 10 ml./minute					
90	Empty bladder	...	...	...	...	...
98	12.5	...	364	15.7	+3.2	111
105	10.1	290	376	13.1	+3.0	99
113	10.0	...	376	12.9	+2.9	97
				Average.....	+2.4	96
<i>3/8/57—23 Hours Hydropenia—300 mg. Cortisone Acetate/Day for 3 Days</i>						
0	300 ml. intravenous 15% mannitol, infusion 15% mannitol 4 ml./minute					
25	Empty bladder	...	...	...	...	...
32	5.6	...	428	8.3	+2.7	125
41	7.0	290	386	9.3	+2.3	142
47	6.3	...	444	9.7	+3.4	112
75	150 ml. intravenous 15% mannitol, infusion 15% mannitol 6 ml./minute					
84	Empty bladder	...	...	...	...	...
84	8.4	...	444	12.9	+4.5	111
91	8.1	...	420	11.7	+3.6	80
97	9.1	290	384	12.0	+2.9	121
103	8.1	...	420	11.7	+3.6	87
				Average.....	+3.3	111
<i>3/28/56—23 Hours Hydropenia—20 Units Pitressin Tannate Intramuscularly at Beginning of Period of Water Deprivation</i>						
0	300 ml. intravenous 15% mannitol, infusion 15% mannitol 6 ml./minute					
20	Empty bladder	...	...	...	...	...
33	10.0	...	400	14.6	+4.6	102
45	7.5	274	444	12.1	+4.6	92
55	7.2	...	396	10.8	+3.6	...
62	6.2	...	408	9.1	+2.9	75
72	5.6	280	420	8.4	+2.8	66
				Average.....	+3.7	86
<i>6/11/57—23 Hours Hydropenia—300 mg. Cortisone Acetate/Day for 7 Days—20 Units Pitressin Tannate Intramuscularly at Beginning of Period of Water Deprivation</i>						
0	300 ml. intravenous 15% mannitol, infusion 15% mannitol 8 ml./minute					
25	Empty bladder	...	...	...	...	...
37	12.2	268	360	16.4	+4.1	64
47	9.5	...	372	13.2	+3.7	63
53	9.6	...	400	14.4	+4.8	63
83	Infusion stopped 30 minutes then continued 6 ml./minute					
93	Empty bladder	...	...	...	...	...
93	3.3	...	488	6.0	+2.7	50
104	3.9	270	484	7.0	+3.1	53
115	5.2	...	428	8.2	+3.0	61
124	4.1	270	440	6.7	+2.6	70
130	7.0	...	460	11.9	+4.9	80
				Average.....	+3.6	63



TABLE II  
PATIENT M. M.

Time (min.)	V (ml./min.)	POsm (mOsm./L.)	UOsm (mOsm./L.)	Osmolal Clearance (ml./min.)	T <sub>H<sub>2</sub>O</sub> <sup>c</sup> (ml./min.)	Creatinine Clearance (ml./min.)
1/14/58—23 Hours Hydropenia						
0	300 ml. intravenous 15% mannitol, infusion 15% mannitol 10 ml./minute					
30	Empty bladder	...	...	....	....	..
38	18.5	...	280	17.8	-0.7	..
48	14.0	...	284	13.7	-0.3	60
58	16.1	289	260	14.5	-1.6	55
64	15.5	...	275	14.8	-0.7	77
Infusion stopped 20 minutes then continued at 8 ml./minute						
104	Empty bladder	...	...	....	....	..
112	10.7	...	308	11.2	+0.5	64
119	10.0	300	324	10.8	+0.8	58
135	10.9	...	312	11.4	+0.5	68
136	200 milliunits Pitressin intravenously, infusion Pitressin 150 milliunits/hour with 15% mannitol 8 ml./minute					
151	10.0	...	380	12.3	+2.3	77
167	10.2	...	388	12.7	+2.5	81
184	10.0	308	392	12.7	+2.7	73
194	9.0	...	380	11.1	+2.1	68
Average before Pitressin.....					-0.2	63
Average after Pitressin.....					+2.4	75
1/23/58—23 Hours Hydropenia—300 mg. Cortisone/Day for 8 Days						
0	300 ml. intravenous 15% mannitol, infusion 15% mannitol 10 ml./minute					
35	Empty bladder	...	...	....	....	..
46	14.5	...	370	16.8	+2.3	94
56	10.4	320	400	13.0	+2.6	68
58	Stop mannitol infusion 20 minutes then continued at 6 ml./minute					
88	Empty bladder	...	...	....	....	..
98	9.5	...	392	11.6	+2.1	60
111	7.9	320	404	9.9	+2.0	66
124	6.6	...	420	8.7	+2.1	59
130	200 ml. intravenous 15% mannitol, infusion 15% mannitol 8 ml./minute					
150	Empty bladder	...	...	....	....	..
158	13.2	...	404	16.9	+3.7	76
168	13.0	318	404	16.5	+3.5	79
169	200 milliunits Pitressin intravenously, Pitressin infusion 150 milliunits/hour with mannitol infusion 8 ml./minute					
181	10.1	...	420	12.8	+2.7	..
195	8.8	...	466	12.4	+3.6	61
210	8.7	330	470	12.4	+3.7	62
219	12.8	...	440	17.0	+4.2	76
Average before Pitressin.....					+2.6	71
Average after Pitressin.....					+3.5	66

considerable rise in the daily urinary volumes resulting from cortisone administration is clearly independent of alterations in urinary solute excretion, which remained materially unchanged throughout. The administration of Pitressin was likewise associated with only minor

alteration in solute excretion. The relative constancy of the latter is reflected in the inverse relationship between the osmolality of the twenty-four-hour urine and its volume.

These results establish the fact that the polyuria resulting from cortisone in these pa-

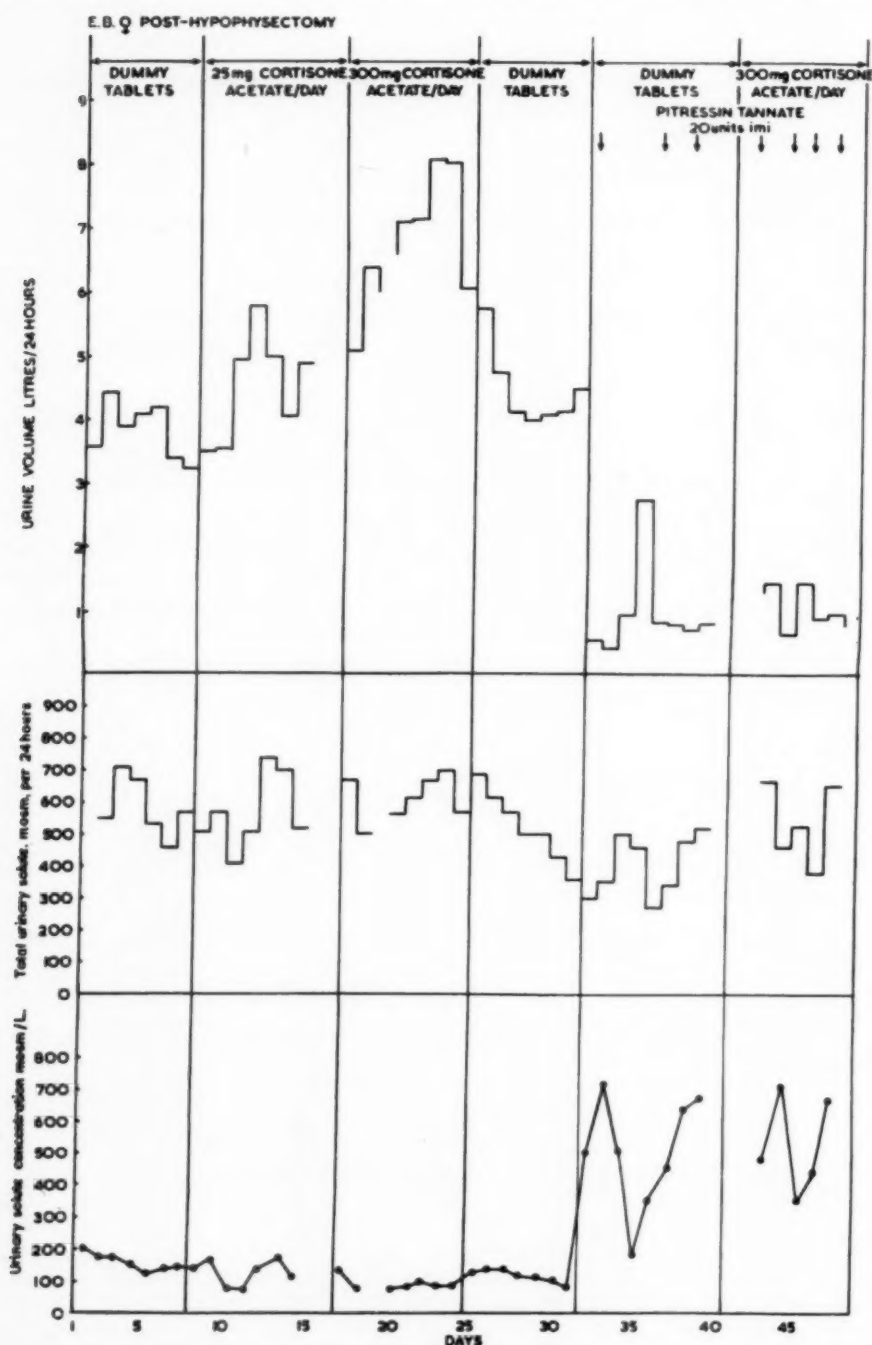


FIG. 1.

tients cannot be explained in terms of an increase in the magnitude of the solute load excreted in the urine. In addition they show that this polyuria and polydipsia is controlled by the administration of Pitressin.

*The Capacity of the Distal Concentrating Mechanism.* *U/P osmolal ratio:* Table III shows the osmolalities of urine and plasma, the ratios of the osmolalities of the urine to those of the plasma (U/P ratio), the rate of urine flow per

minute and the clearance of creatinine following twenty-two hours of food and water deprivation in both patients. During the administration of dummy tablets the U/P ratio averaged 1.47 and 1.58 for E. B. and M. M., respectively. The administration of 300 mg. of cortisone for three days to E. B. and for eight days to M. M., which produced severe polydipsia and polyuria, did not result in a fall in this ratio. A slight rise to 1.71 occurred in Case I (E. B.), whereas there

was no change in Case II (M. M.). In both subjects the U/P ratio was significantly increased to 2.55 and 2.70 when 20 pressor units of pitressin tannate was given by intramuscular injection at the beginning of another test period. The Pitressin-induced increase in U/P osmolar ratio occurred whether cortisone was being given or not.

*The reabsorption of solute-free water following hydropenia during osmotic diuresis:* Figures 3 and 4 show the results obtained in the two patients during osmotic diuresis induced by the infusion of mannitol. Protocols of these experiments from which the figures are constructed are given in Tables I and II. In the figures the osmolar clearance  $UV/P$  is plotted against the urine flow for each clearance period. After twenty-three hours deprivation of food and water during the administration of dummy tablets both patients produced urine which was almost isosmotic with plasma. The points representing individual clearance periods are seen to be distributed around the isosmotic (1:1) line. This indicates that under these conditions there was little reabsorption or excretion of solute-free water during the diuresis. The average value for  $T_{H_2O}^C$  was calculated from all the clearance periods in both patients and amounted to  $-0.1$  and  $-0.2$  ml./minute for E. B. and M. M., respectively. Following the administration of 300 mg. cortisone per day for three days to E. B. and for eight days to M. M., the osmolar clearance was markedly increased for any given rate of urine flow and the points representing the clearance periods were deviated to the left of the isosmotic line. This indicates a considerable degree of tubular reabsorption of water which on the average became  $+3.3$  ml./minute for E. B. and  $+2.6$  ml./minute for M. M. An intermediate value was obtained for E. B. following the administration of 25 mg. cortisone for seven days. An essentially similar value for  $T_{H_2O}^C$  was obtained for E. B. when 20 units of pitressin tannate were injected at the beginning of the period of dehydration, whether cortisone was being given or not. In the case of M. M. to whom Pitressin was given by infusion, the average value for  $T_{H_2O}^C$  obtained with Pitressin alone was similar to that which resulted from the administration of cortisone. The giving of Pitressin during the diuresis induced by treatment with cortisone for seven days resulted in a further slight rise in the average value of  $T_{H_2O}^C$  from  $+2.6$  ml./minute to  $+3.5$  ml./minute.

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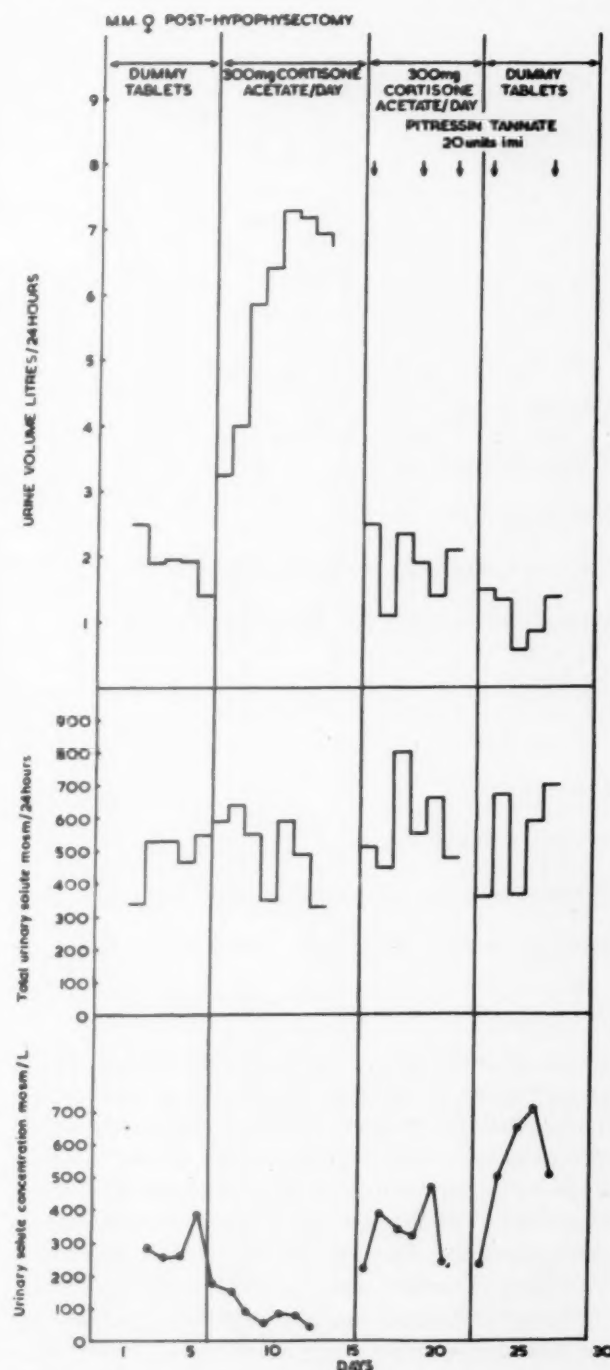


FIG. 2.

#### COMMENT

*Nature of the Cortisone-induced Polyuria.* Our observations show that in these two patients the administration of cortisone rapidly induces severe polyuria and polydipsia and that this effect occurs in the absence of significant alteration in the excretion of urinary solute. The changes in urinary output, therefore, cannot be ascribed to variations in solute excretion and



TABLE III  
PLASMA AND URINE OSMOLALITIES, RATE OF URINE FLOW AND CREATININE CLEARANCE  
AFTER TWENTY-TWO HOURS OF FOOD AND WATER DEPRIVATION

Therapy	Osmolality (mOsm./L.)		Urine Flow V (ml./min.)	U/P Ratio	Creatine Clearance (ml./min.)
	Plasma	Urine			
<i>Patient E. B.</i>					
Dummy tablets . . . . .	294	426	0.32	1.45	44
...	...	436	0.35	1.48	53
25 mg. cortisone acetate . . . . .	288	488	0.45	1.69	72
...	...	524	0.46	1.81	82
300 mg. cortisone acetate . . . . .	284	476	0.41	1.68	73
...	...	496	0.38	1.74	66
Pitressin tannate, no cortisone acetate . . . . .	263	632	0.31	2.40	..
...	...	672	0.53	2.55	80
Pitressin tannate plus 300 mg. cortisone acetate . . . . .	262	660	0.50	2.52	62
...	...	684	0.75	2.61	63
<i>Patient M. M.</i>					
Dummy tablets . . . . .	280	456	0.63	1.63	60
...	...	424	0.60	1.53	62
300 mg. cortisone acetate . . . . .	304	480	0.59	1.58	66
...	...	486	0.60	1.60	73
Pitressin tannate, no cortisone acetate . . . . .	280	752	0.42	2.68	58
...	...	760	0.43	2.70	70
Pitressin tannate plus 300 mg. cortisone acetate . . . . .	270	690	0.51	2.56	62

demand further explanation. The occurrence of progressive dilution of the urine in association with the polyuria induced by cortisone suggested the possibility of an aggravation of any existing deficiency of antidiuretic hormone. It seems reasonable to suppose that both of our patients had sustained damage to the base of the brain resulting in some impairment in ADH secretion. E. B. suffered from moderate polyuria even when no cortisone was being given and in both patients the U/P osmolal ratio following twenty-two hours of water deprivation was low and was significantly increased when Pitressin was given. This effect is not seen in normal people with intact supraopticoneurohypophyseal systems.

Aggravation of any existing deficiency in the secretion of antidiuretic hormone would be expected both to reduce the U/P osmolal ratio obtained after hydropenia and to produce a significant increase in the volume of solute-free water excreted during osmotic diuresis. The U/P osmolal ratio was not reduced by cortisone in

either subject so that deterioration in concentrating power cannot account for the polyuria. Furthermore, under conditions of osmotic diuresis cortisone appeared to have the effect, usually accepted as a characteristic of ADH, of promoting the reabsorption of solute-free water. Both observations are inconsistent with the view that the polyuria induced by cortisone is of the nature of diabetes insipidus or that it is due to a direct action of cortisone on the renal tubules, reducing their capacity to reabsorb solute-free water. The conclusion that, whatever the degree of ADH insufficiency in these two patients, the polyuria induced by cortisone is the consequence of polydipsia, seems inescapable.

Little is known about the mechanisms in the body which control water intake but the evidence that an essential thirst centre is located in the hypothalamus, at least in the goat, has recently been reviewed [19]. In these animals stimulation of the anterior part of the "drinking area" was found to cause both polydipsia and

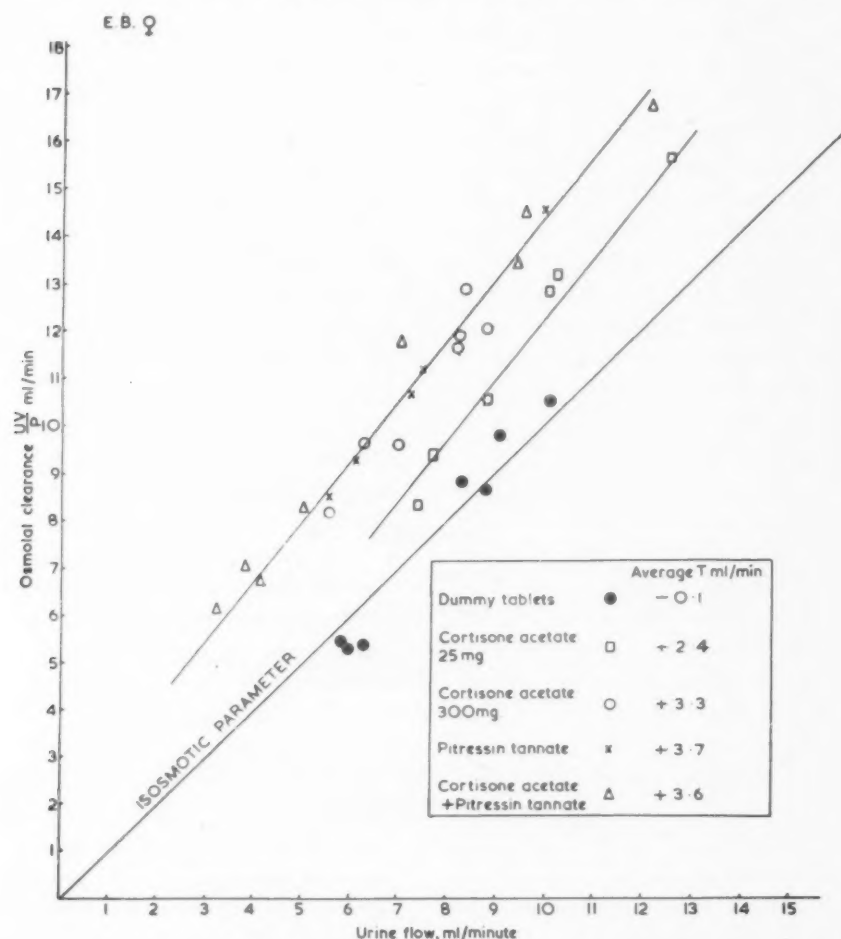


FIG. 3.

antidiuresis of the neurohypophyseal type while stimulation of the posterior part produced polydipsia alone. The physiological stimuli to these centres are unknown and although they were activated in the experimental preparation by hypertonic saline solution the possibility that this was a non-specific effect cannot be excluded. In the present state of knowledge it is impossible to be sure of the mechanism by which cortisone stimulated the desire for water in our two patients. It may be that this is achieved by direct pharmacological action on that part of the centre corresponding to the posterior zone in the goat hypothalamus. Such an effect of cortisone on the hypothalamic nuclei cannot be dependent solely upon the absence of ADH in the body or a significant diminution in its amount, since primary polydipsia was not a feature of the single patient with anterior and posterior pituitary deficiency reported by Leaf *et al.* [2]. Unlike our patients, however, this patient had not suffered surgical interference at the base of the brain.

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Neither was primary polydipsia a feature of the patient suffering from anterior and posterior pituitary deficiency reported by Engstrom and Liebman [20]. The administration of cortisone to their patient, however, resulted in serum sodium concentrations between 155 and 160 mEq./L. and these authors postulated a defective response of thirst to this plasma osmolality.

An alternative explanation is that cortisone induces thirst by increasing the renal conservation of sodium out of proportion to that of water which, in the presence of ADH deficiency, continues to be excreted in the urine. Elevation of the concentration of serum sodium which would then occur might be responsible for increasing the desire for water. It is difficult to accept this explanation, which was originally advanced by Stribling and Spurr [5], in the face of the demonstration that cortisone does not diminish the renal power to conserve water during hydropenia as evaluated by the U/P ratio attained after twenty-two hours of water deprivation, and

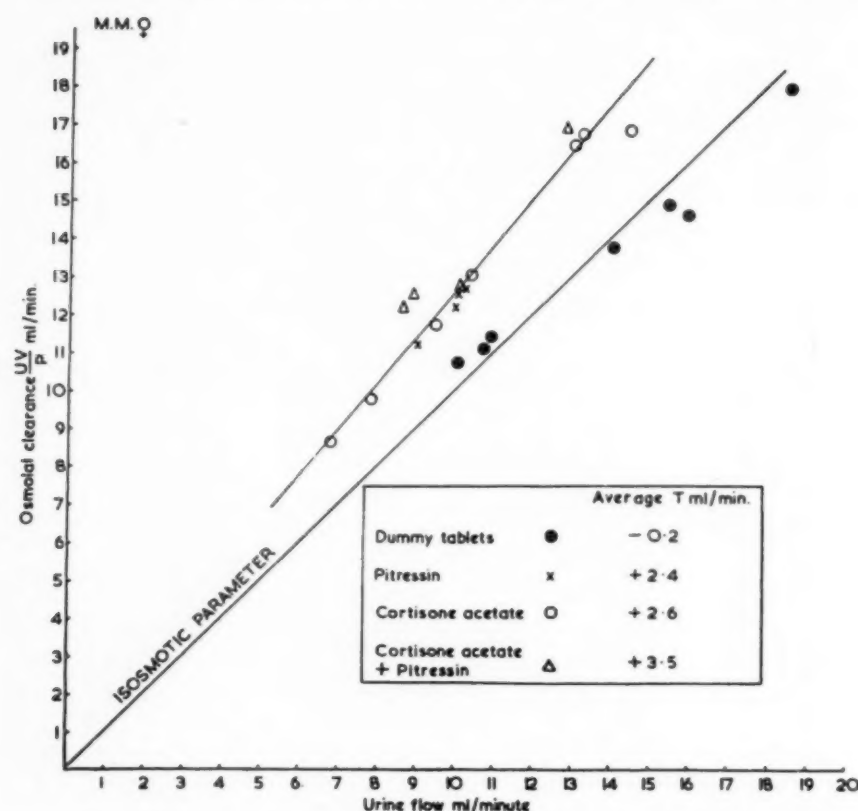


FIG. 4.

increases the amount of solute-free water reabsorbed during osmotic diuresis. The concentration of serum sodium in one of our patients (M. M.) during treatment with cortisone was distinctly higher than when she was receiving dummy tablets but was nevertheless within normal limits.

From this study and from consideration of the data reported in the literature, in which there appears to be an association between the administration of cortisone and the occurrence of polyuria, the mechanisms responsible for the syndrome seem to be twofold.

(1) In the presence of ADH insufficiency cortisone may induce polyuria by stimulating the appetite and increasing the solute load in the urine. The polyuria is then merely an expression of a given degree of diabetes insipidus and is analogous to the experimental observation of Winter *et al.* [3] and Beaser [4]. This appears to have been the sole mechanism of action in the single patient reported by Leaf *et al.* [2].

(2) Following hypophysectomy cortisone induces polyuria by stimulating thirst with consequent polydipsia. This appears to have been the sole mechanism of action demonstrated in our own two patients, and in that of Stribling and

Spurr [5]. It may have been the mechanism involved in the patient reported by Skillern *et al.* [6] but the data are not sufficiently extensive to allow this conclusion to be reached with certainty.

It is impossible from the published results of Ikkos *et al.* [7] to decide which of these two mechanisms was responsible for the polyuria in their patients, for in these studies the solute load was not controlled during the administration of cortisone.

Neither the published records, including that of Ikkos *et al.* [7], nor our own results indicate that any alteration in the severity of ADH deficiency can explain the occurrence of the polyuria. For this reason, although ADH deficiency is presumably present, it is inappropriate to refer to the syndrome following hypophysectomy as diabetes insipidus.

*Action of Cortisone on the Distal Concentrating Mechanism.* It remains to discuss the effect produced by cortisone on the renal concentrating mechanism. The administration of the hormone had the paradoxical effect of increasing the volume of water capable of being reabsorbed during osmotic diuresis, while at the same time producing polyuria and polydipsia. We have observed a



similar effect on the reabsorption of solute-free water in a number of patients suffering from adrenocortical insufficiency in whom there was no damage to the base of the brain and in whom no polydipsia or polyuria was induced when cortisone was given [27]. This effect does not occur in normal persons [21,22].

The possibility that cortisone and other adrenocorticoids influence the renal mechanisms concerned with the excretion of water as distinct from electrolytes has been the subject of numerous investigations. The majority of these studies have been carried out in man and in animals during water diuresis. It has been shown in these circumstances that cortisone increases the capacity of the body to eliminate a water load and exerts an effect which would appear to be antagonistic to the antidiuretic hormone [23-25]. Raisz *et al.* [22] have recently suggested that in normal man this effect is brought about by a direct action on the kidney, either by increasing the reabsorption of sodium by the distal tubule or by diminishing the distal tubular reabsorption of water. Their studies, and those of others carried out under conditions of severe water diuresis, may be assumed to provide information about the action of cortisone in the presence of small amounts of ADH or in its complete absence, when the excretion of solute-free water is of considerable magnitude,  $T_{H_2O}^C$  being negative. Skillern *et al.* [6] likewise showed in acute experiments that hydrocortisone administered intravenously induced a slight increase in solute-free water excretion in a patient with severe diabetes insipidus and Addison's disease in whom the value of  $T_{H_2O}^C$  was  $-4.95$  ml./minute. We have had a similar experience with one case of uncomplicated diabetes insipidus in which the administration of cortisone appeared to change the value of  $T_{H_2O}^C$  by  $-1.0$  ml./minute.

There are certain older observations in the literature which suggest that the adrenocorticoids may be concerned in the production of urine more concentrated than plasma and therefore in the tubular reabsorption of water. In 1939 Wilson and Sunderman [26] were able to demonstrate salt retention in patients with Addison's disease given a high sodium intake and subjected to water restriction. They attributed this to a limitation in the concentrating power of the kidney. Similarly, Reforzo-Membrives, Power and Kepler [27] showed impairment in concentrating ability in patients suffering from Addison's disease following

comparable periods of water deprivation. More recently Burnett [28] demonstrated the inability of patients suffering from Addison's disease to increase the U/P ratio for sodium after the administration of hypertonic saline solution and this might also be interpreted as indicating reduced tubular reabsorption of water. These observations were made under conditions in which the value of  $T_{H_2O}^C$  was positive and in which circulating ADH is likely to have been present.

The conditions under which our own observations were carried out were such as are known to induce maximal activity of the concentrating mechanism in normal persons [29]. Nevertheless, when neither cortisone nor Pitressin was given the U/P osmolal ratio of both patients was low and the value for  $T_{H_2O}^C$  virtually zero. Whereas the administration of Pitressin restored both these functions towards normal, cortisone increased the value for  $T_{H_2O}^C$  only when the solute excretion was increased by infusion with mannitol. It had virtually no effect on the U/P osmolal ratio at basal rates of solute excretion. This effect of cortisone could be due either to a direct action on the renal concentrating mechanism itself or to the augmentation of this process by an increase in the effectiveness or amount of circulating ADH.

Existing experimental and clinical evidence lends little support to the view that cortisone stimulates ADH secretion directly or indirectly or that it augments its activity. Gaunt [30] has recently shown that hydrocortisone causes an increase in the amount of ADH in the posterior pituitary and that it prevents depletion of neurosecretory material in the neurohypophysis in both intact and adrenalectomised rats. Guinsberg [31] showed the half-life of vasopressin in the blood of rats to be doubled after adrenalectomy and salt depletion. Likewise Dingman and Despontes [32] demonstrated in both normal man and patients suffering from Addison's disease that the administration of cortisone and hydrocortisone elevated the dose of nicotine required to stimulate ADH secretion, the antidiuretic response to hypertonic saline solution being unaltered by adrenal steroids. Dingman and Thorn [33] reported a series of patients with diabetes insipidus in whom the administration of cortisone abolished the antidiuretic effect of nicotine without altering the renal sensitivity to Pitressin. These observations suggest that cortisone inhibits ADH secretion from the neurohypophysis. The absence of a significant effect

of cortisone upon the U/P ratio in our own patients is in any case unlike that of ADH activity. The failure of cortisone to alter the U/P ratio is seen also in the patients studied by Ikkos *et al.* [7] and by Stribling and Spurr [5].

This raises the interesting possibility that under conditions of osmotic diuresis in which increased amounts of sodium and water are delivered to the distal tubule, cortisone itself acts directly on the renal concentrating mechanism, increasing the reabsorption of solute-free water. There is evidence which suggests that the function of ADH in the renal tubules, as in the frog's skin, is to increase the permeability of the tubule to the movement of water along osmotic gradients rather than the creation of these gradients themselves [34-36]. In this event it is necessary to invoke a separate mechanism for water reabsorption which, although dependent upon this permissive action of ADH, is not itself due to it. Berliner and Davidson [37] have recently shown that in dogs under conditions of water diuresis, reduction in the rate of glomerular filtration in one kidney induced an osmolality in the urine from this kidney slightly higher than that of the plasma, the urine being produced simultaneously by the unaffected kidney remaining less concentrated than plasma. These workers suggest that alterations in the amount of water and sodium reaching the concentrating segment may themselves influence the amount of water reabsorbed and it is therefore probable that factors which alter the reabsorption of sodium at this site are important.

The countercurrent hypothesis of Wirz [38] provides a mechanism by which, in the presence of ADH, sodium reabsorption may be directly linked with solute-free water reabsorption. Sodium reabsorption in the loop of Henle is considered to produce an increase in the osmolal concentration in the interstitial tissues around the loop. In the presence of ADH, which equalises osmotic gradients, this increase in concentration would result in water absorption from the anatomically adjacent collecting ducts. If this hypothesis is true in man, alterations in sodium reabsorption by the ascending limb ought to be accompanied by alterations in urinary osmolality and in the value of  $T_{H_2O}^c$  in the presence of ADH. Our observations are in accord with this view.

It is suggested that the administration of cortisone to such patients as we have described increases distal tubular reabsorption of sodium. In the presence of subnormal ADH secretion and

therefore of some impairment of tubular permeability to water, the added stimulus of sodium reabsorption induced by cortisone during osmotic diuresis results in additional reabsorption of water with consequent rise in  $T_{H_2O}^c$ . The limiting factor to the establishment of a normal U/P ratio after water deprivation in these two cases would appear to be the permeability of the tubule to water migration and this ceases to be limiting when Pitressin is administered. On the other hand the limiting factor to the establishment of a normal value of  $T_{H_2O}^c$  in conditions of osmotic diuresis which supplies the distal tubule with increased amounts of sodium would appear to be the osmotic gradient determined by the amount of sodium reabsorption, and this ceases to be limiting when cortisone is given.

These explanations of the effect of cortisone in our patients receive some experimental support from the results recently reported by Guinnebault and Morel [39]. They have shown in salt-loaded rats that the ratio of the concentration of sodium in the papilla of the kidney to that of the plasma is reduced from 1.3 to 0.91 by adrenalectomy, and in rats given a water load this ratio is raised from 0.6 to 0.92. These results confirm Wirz's demonstration of the existence of a concentration gradient between the papilla and the plasma when the urine is hypertonic and suggest further that the establishment of this gradient is dependent upon the presence of adrenocorticoids.

This view of the action of cortisone is not inconsistent with the failure to demonstrate alteration in  $T_{H_2O}^c$  in normal hydropenic subjects or with the demonstration that cortisone increases solute-free water excretion in diabetes insipidus or in the absence of circulating ADH. In the former, with maximum tubular permeability induced by ADH after hydropenia, cortisone may be considered not to increase significantly the amount of sodium already being reabsorbed under the influence of the subject's endogenous adrenocorticoids. In the latter, the absence of tubular permeability to water does not permit water reabsorption irrespective of the amount of sodium reabsorption occurring in the ascending limb of the loop of Henle.

This hypothesis regards the establishment of a normal U/P osmolal ratio after water deprivation and a normal value for the reabsorption of solute-free water during osmotic diuresis as being the result of a balance of factors which include ADH and the extent of sodium reabsorption. It seems likely that the latter is determined to some

extent by adrenocorticoids and cortisone brings about the effect we have observed by altering its magnitude.

At the present time such a view remains hypothetical but our own results and certain other experimental observations would seem to be best explained within its framework.

#### SUMMARY

1. A study has been made of the nature of the polyuria induced by cortisone occurring in two patients who had undergone hypophysectomy.

2. In these two patients the polyuria has been shown not to be due to an increase in the load of urinary solute nor to deterioration in those renal functions concerned with water conservation.

3. The view that the phenomenon is due to primary polydipsia seems inescapable. A review of the literature suggests that this mechanism is probably responsible for the syndrome in similar patients described by other workers, with the exception of the patient reported by Leaf et al. [2] in whom alteration in solute load determined the increase in urinary volume. In these circumstances it is inappropriate to refer to the syndrome as diabetes insipidus.

4. In our patients the administration of cortisone has been shown to improve the renal capacity to absorb solute-free water during osmotic diuresis under conditions of hydropenia.

5. This effect is discussed in the light of the Wirz countercurrent hypothesis and in relation to the view that the action of ADH is one of increasing the permeability of the renal tubules to the movement of water along osmotic gradients. The increase in solute-free water reabsorption brought about by cortisone might then be explained by the effect of the steroid in augmenting reabsorption of sodium.

*Acknowledgment:* We are indebted to Dr. Albert Recht for carrying out the chemical determinations of mannitol and to Professor D. M. Dunlop for his permission to study the patients under his care.

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# Primary Lymphosarcoma of the Stomach\*

## *A Clinical Study of Seventy-five Cases*

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**E**IGHTY-FIVE patients with primary lymphosarcoma of the stomach were admitted to the Mount Sinai Hospital in the years 1934 to 1954. During this period there were 2,854 cases of gastric neoplasm. This represents an incidence of 3.4 per cent of all tumors of the stomach. Various authors [1-3] have reported an incidence of sarcoma of the stomach ranging between 1.5 and 2.5 per cent of all gastric neoplasms, 30 to 50 per cent of which were lymphosarcomas. Most reports agree that lymphosarcoma constitutes the largest single group of gastrointestinal sarcomas. While various subheadings of lymphosarcoma have been described, the most frequent subdivision employed has been that limited to reticulum cell sarcoma, or large round cell lymphosarcoma, and small round cell lymphosarcoma, also sometimes called malignant lymphocytoma [4]. The present series is limited to this classification, namely, small round cell lymphosarcoma and reticulum cell sarcoma; Hodgkin's disease, leiomyosarcoma and other modifications of lymphomatous tumors are not included.

Ten of the patients have been lost to follow-up and the remainder, seventy-five patients with primary lymphosarcoma of the stomach, constitute the basis of this report. This total includes sixty-four patients with small round cell lymphosarcoma and eleven with reticulum cell lymphosarcoma who were followed up for a period of twenty years. The diagnosis in each case was made by pathologic examination of the resected specimen.

Although many authors consider involvement of the stomach as *prima facie* evidence of primary disease, lymphosarcoma was considered primary in the stomach only when the complaints were exclusively or predominantly gastrointestinal and the lesion was limited to the stomach or appeared to originate there. Patients in whom the

gastric tumor appeared to have developed secondary to involvement of other areas or contiguous tissues are not included in this report. Where the differences between reticulum and small cell lymphosarcoma have been particularly significant we have separated the material to analyze the results in each group. However, because of the small number of reticulum cell sarcomas these figures may not be clinically significant.

**Clinical Findings.** The average age in this series was fifty-eight years. The youngest patient was twenty-eight years of age and the oldest seventy-three. (Table I.) The average age of patients with reticulum cell sarcoma was the same—fifty-eight years. The sex ratio was forty-five males to thirty females or 1.5:1. The group includes one male Negro, the only non-Caucasian.

The average duration of symptoms for the entire series was nine months, varying from two weeks to four years. The most common complaint was abdominal pain; this was present in sixty-three patients. (Table II.) The pain was diffuse or located high in the epigastrium, the right upper quadrant or peri-umbilical region with occasional radiation to the right shoulder, rarely to the inguinal region. In eight patients the pain was ulcer-like, aggravated by hunger

TABLE I

Age (decades)	Cases (No.)
Third . . . . .	1
Fourth . . . . .	6
Fifth . . . . .	18
Sixth . . . . .	23
Seventh . . . . .	21
Eighth . . . . .	6
Total . . . . .	75

\* From the Department of Medicine and the Gastrointestinal Clinic, The Mount Sinai Hospital, New York, New York

TABLE II

Symptoms	Cases (No.)
Pain.....	63
Abdominal.....	45
Epigastric.....	13
Upper right quadrant.....	5
Radiating to right shoulder.....	3
Relieved by food.....	8
Bleeding.....	21
Melena.....	16
Hematemesis.....	7
Nausea or vomiting.....	19
Anorexia.....	10
Dyspepsia, abdominal pressure or distress.....	8
Dysphagia.....	6
Fever.....	3
Diarrhea.....	3
Glossitis.....	1
Weight loss (greater than 10 lb.).....	15

and relieved by food or antacids. Abdominal discomfort due to dyspepsia, belching, nausea or non-specific gastrointestinal complaints was described by twenty-three patients, but in none of these was any one symptom a constant feature. Vomiting was complained of by fourteen patients, but in only two was it an important symptom. Dysphagia was present in six patients and appeared to be related to involvement of the esophagus or cardia. Diarrhea was present in three instances. Evidences of gastrointestinal bleeding were found in twenty-one patients; hematemesis in seven, melena in twelve and hematemesis and melena in two. Weight loss was a prominent factor in fifteen patients and varied from 10 to 50 pounds, in some accounting for as much as a 35 per cent reduction in weight. Fever was present in three patients; in view of the tremendous size of many of these lesions and the amount of necrosis found in the specimens, it is surprising that pyrexia was present only in this small number.

The past history in three patients was significant. Abdominal surgery for perforated ulcer had been performed (elsewhere) from three to nine months prior to their present admission.

Physical examination was contributory in thirty patients, a mass being present in the right upper or left upper quadrant. (Table III.) In addition, hepatomegaly was found in sixteen patients and splenomegaly in eight. Abdominal tenderness in the epigastrium or right upper quadrant was present in eight patients. Jaundice

TABLE III

Physical Findings	Cases (No.)
Mass (right or left upper quadrant or epigastric).....	30
Hepatomegaly.....	16
Splenomegaly.....	8
Tenderness (epigastric or right upper quadrant).....	8
Pleural effusion.....	3
Jaundice.....	1

was observed in only one subject. A left pleural effusion was seen during the course of the disease in three patients.

Rehbus test meals were performed in thirty-seven patients. (Table IV.) In twenty-five patients free hydrochloric acid ranging from 15 to 70 mEq./L. was found, the majority presenting more than 50 mEq./L. Histamine anacidity was recorded in twelve patients. Guaiac-positive gastric contents were recorded in three patients and guaiac-positive stools in twenty. Severe anemia (less than 7 gm. per cent), usually hypochromic microcytic in nature, was found in nine patients. Monocytosis was present in two patients, in one case seven per cent and in the other 11 per cent; these were the only unusual blood counts recorded. In four patients bone marrow aspiration was performed; one was reported as osteosclerotic anemia and three were normal.

Roentgenographic diagnoses were available in sixty-four of the seventy-five patients. Many of the barium meal examinations were not made at this hospital although the films were available to us. The diagnoses are recorded over a span of twenty years. In a historical sense it is interesting that carcinoma was diagnosed thirty times, carcinoma or hypertrophic rugae four times,

TABLE IV

Laboratory Findings	Cases (No.)
Free hydrochloric acid (15-70 mEq./L.)....	25*
Guaiac-positive stool.....	20
Guaiac-positive gastric contents.....	3
Anemia (less than 7 gm.).....	9
Monocytosis.....	2
Osteosclerotic anemia.....	1

\* Performed in thirty-seven patients.



TABLE V

Location of Lesion	Cases (No.)
Corpus.....	21
Anterior and posterior wall.....	9
Posterior wall.....	6
Greater curvature.....	4
Lesser curvature.....	2
Antrum.....	20
Diffuse.....	15
Pylorus.....	10
Antrum and pylorus.....	5
Cardia.....	4
Esophagus.....	3
Duodenum.....	7

hypertrophic rugae twice, cancer and gastric ulcer four times, gastric tumor three times, perforated peptic ulcer of the duodenum or stomach three times, gastric ulcer five times, duodenal ulcer three times and cardiospasm once. Lymphosarcoma as a definitive diagnosis was recognized six times and as a presumptive diagnosis in nine additional cases. Seventy-five per cent of the diagnoses of lymphosarcoma were made within the past four years.

Gastroscopy was performed in sixteen patients and esophagoscopy in four patients in this series. Carcinoma was diagnosed ten times, cancer of the stomach or lymphosarcoma twice, gastric ulcer twice and lymphosarcoma six times.

Surgery was performed on all the patients in this series. Subtotal gastrectomy was the procedure in thirty-eight patients, subtotal gastrectomy and duodenectomy in seven, and esophago-gastrectomy in six patients. In twelve, a total gastrectomy was performed. In another twelve patients only a biopsy of the lesion during laparotomy was obtained; in three of these, esophagoscopy biopsy also was performed. Jejunostomy for feeding purposes was carried out in seven (these were older records) and colectomy because of extension of the disease in one. The first portion of the duodenum was removed because of disease in seven patients, a left lobe hepatectomy performed in one patient, subtotal pancreatectomy in one and splenectomy in ten.

*Pathology.* The lesion was located in the corpus in twenty-one patients, involving the anterior and posterior walls, the posterior wall, the greater curvature and lesser curvature, in that order of frequency. (Table v.) The antrum

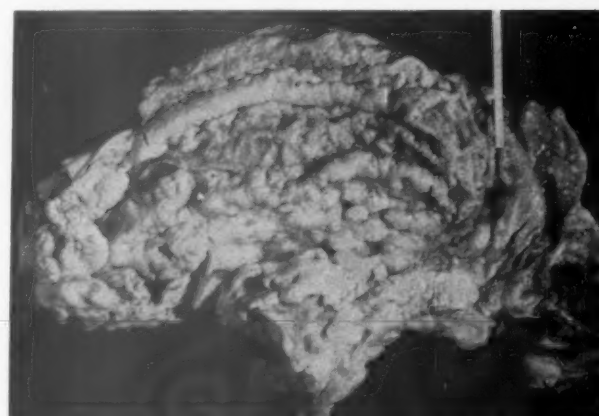


FIG. 1. CASE 50. The stomach is infiltrated by a huge flat lesion. A 5 cm. ulceration is present in its center. (Ulcerative.)

TABLE VI

Gross Pathology	Cases (No.)
Infiltrative.....	14
Ulcerative (flat).....	33
Nodular (multiple).....	14
Polypoid.....	6
Combined.....	8
Total.....	75

was involved in twenty patients, the pylorus in ten, the antrum and pylorus were both involved in five, and the cardia in four patients. The lesion was classified as diffuse in fifteen cases, i.e., when more than 50 per cent of the stomach was involved. Finally, in addition to the stomach the esophagus was involved in three patients and the duodenum in seven.

Pathological examination of the resected specimens revealed that the lesions can be divided grossly into five forms (Table vi): infiltrative, ulcerative, nodular, polypoid and combined. These represent, it seems, progressive stages in the growth of the neoplasm. The first is a localized or diffusely infiltrative lesion presenting thickened, prominent rugae and possessing a smooth or slightly granular or cobblestoned surface, occasionally with minimal superficial ulceration and simulating the so-called hypertrophic gastritis. (Fig. 1.) Less frequently the summits of the involved rugae presented a more flattened appearance producing the effect of plaques. Occasionally the appearance is that of a flat, annular, red, rubbery mass. The presence of diffuse disease did not necessarily imply that the

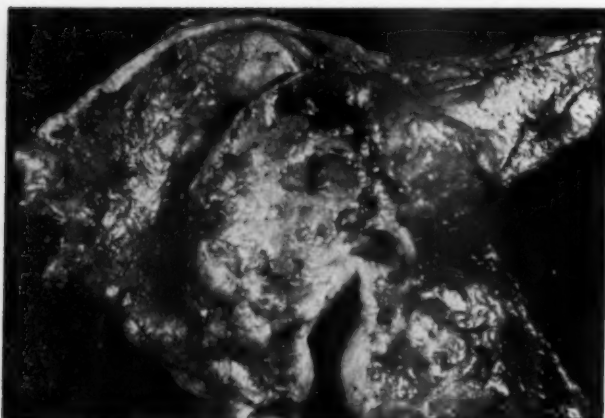


FIG. 2. CASE 55. At the pyloric end of the specimen is a huge, irregular mass that completely encircles the lumen of the stomach. The mass presents three deep, irregular ulcerations 1 to 3 cm. in diameter, perforating through the walls of the stomach to large extragastric masses on the lesser curvature. (Polypoid.)

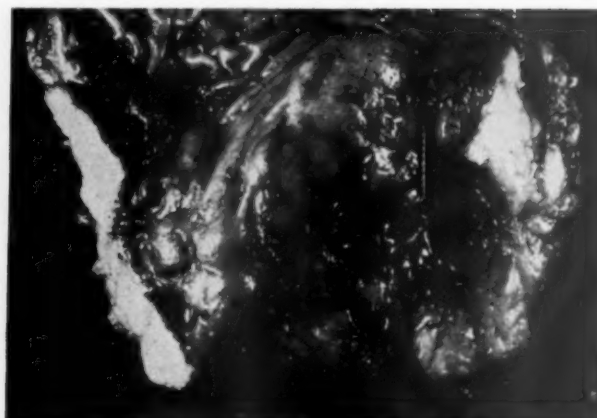


FIG. 3. CASE 29. The specimen reveals an infiltrative tumor with elevated margins involving both anterior and posterior walls. Only superficial ulceration is present and this can be visualized in the lower half of the specimen. The serosa presented scattered small grayish nodules as well as larger hemorrhagic nodules. (Combined.)

lesion extended in continuity. Multiple areas of involvement occasionally were separated by free intervals of normal appearing tissue. This is the category that has been most frequently described as pathognomonic of lymphosarcoma; actually, this type was present in only fourteen cases (18 per cent).

The most common form was a localized tumor presenting as a shallow saucer-like ulcerating lesion, seen in thirty-three patients (42 per cent). The ulcerations varied from 3 to 18 cm. in diameter with necrotic, grayish or yellowish bases and raised indurated edges that make them indistinguishable from carcinoma. (Fig. 2.) However, the rugal folds around the ulceration were thickened and in many instances radiated to the lesion. In five patients more than one flat ulcerating lesion was present.

Multiple intragastric nodular masses, often with subserosal nodules, were found in fourteen patients. The nodules were often scattered over a wide area of the gastric mucosa. They varied in size from 0.5 to 4 cm. in diameter. The nodule is a submucosal tumor and the mucosa over it may be smooth and intact, although superficial or deep ulceration usually is found. Ulceration may occur on the mucosal or the serosal surface of the nodule, most frequently on the former. Between the nodules the gastric rugae often appeared thickened, as in the infiltrative form, but they may be normal or hemorrhagic. Rarely, the nodules were more prominent on the serosa, presenting a light gray hemorrhagic sheen without revealing much mucosal abnormality.

A large polypoid, rubbery, ulcerating mass

was seen in eight patients and in four of these the lesion presented a small intraluminal and a large extraluminal or exogastric component. The largest of these lobulated masses measured 15 cm. in diameter. This lesion was much more vascular than the other types and possessed a spongy texture frequently described in textbooks as typical of lymphosarcoma [5]. This type lesion often developed a deep ulceration that perforated the entire gastric wall, or extended into the mass beyond the contours of the gastric wall. (Fig. 3.) The polypoid form of lymphosarcoma may also produce obstruction when it develops in the prepyloric area of the stomach or in the region of the cardia. Pyloric obstruction was present in three cases and obstruction at the cardia in one. Finally, there is the combined form which presented the pathological development of different forms, apparently *pari passu*: infiltrative thickening, saucer-like ulcerations, nodular masses, and polypoid lesions. (Fig. 4.) This pathological grouping was found in eight instances. In no patient were all of these various forms of the disease found, but in four instances combinations of three different types of lesions were present, the most common being the infiltrative, the saucer-like ulceration and the nodular masses.

Ulceration of the tumor was found in 80 per cent of the specimens. The largest ulcerations were seen in the flat saucer-like tumor. Shallow or superficial ulcerations were found in 30 per cent; this type of ulceration is usually seen in the diffuse, nodular or infiltrative type of lesion. Multiple ulcerations were observed in sixteen

specimens. A polypoid lesion was the seat of a deep penetrating ulcer in six cases.

Although perforation of an intestinal viscus due to lymphosarcoma has been infrequently described [6,7], in this series it occurred in eleven patients or more than 14 per cent. In nine patients the perforation was gastric in origin and in two, duodenal. In two instances a gastric ulcer perforated into another viscus producing in one case a gastroduodenal fistula and in the other a gastroduodenojejunal fistula. In the latter the lesion involved not only the pylorus but the duodenum as well, although the perforation was limited to the first portion of the duodenum. The remainder were perforations of a gastric lesion into the pancreas, gastrosplenic ligament, lesser sac or mesentery. In five instances a walled off gastrosplenic or posterior or anterior gastric wall abscess was found. Invasion of the liver was found in fourteen patients, of the spleen in six. Involvement of the diaphragm or pleura occurred five times in this series. Penetration into the pancreas was found in three patients.

Lymphosarcoma of the stomach is believed to originate in the lymphoid germinal follicles of the submucosa [8,9]. At first, proliferation of cells produces a thickening of the wall and a prominence of the gastric rugae. Because the process is limited to the submucosa, extruding between the tissue planes, there is no mucosal ulceration and scant gross evidence of an underlying pathologic condition. In eight of the seventy-five cases the lesion was confined to the submucosa and mucosa; there was no penetration of the muscularis. Eventually, this diffuse infiltration of the submucosa is distinguished by a more pronounced thickening and some loss of pliability of the gastric wall. In the nodular and polypoid variety, the tumor becomes more cellular and the infiltration more vascular. Nevertheless, lymphosarcoma characteristically evokes little fibroblastic reaction in the surrounding tissue.

The histological diagnosis of lymphosarcoma presents particular problems which are reflected in cytological alterations that apparently take place during the course of the disease. A predominant cell characteristic of one form of lymphoblastic tumor apparently may give way at some later date in the development of the disease to another lymphomatous type. Such instances have been reported in the literature and are well known [10,11]. Multiple diagnoses consequently were made in four cases in this series. In one patient, following a gastric resec-



FIG. 4. Necropsy specimen. The entire stomach is infiltrated. The folds are prominent, markedly thickened, and some, like the ridge along the superior border, more than 1.5 cm. above the mucosal surface; others present beading along their summits. Only one small ulcerated area is present at the left lower margin along the lesser curvature. The duodenum (at right) revealed submucosal infiltration. (Diffuse.)

tion, the diagnosis was small cell lymphosarcoma. Eventually this patient died at another institution of recurrent lymphosarcoma, and at necropsy a diagnosis of Hodgkin's disease was made. In another case following examination of the resected specimen the diagnosis was small cell lymphosarcoma but biopsy of a lymph gland (at another institution) at a later date for evaluation of metastatic nodules was reported as reticulum cell lymphosarcoma. In a third patient with a primary diagnosis of follicular lymphoblastoma the postmortem diagnosis two years later was small round cell lymphosarcoma. In the fourth case a subtotal gastrectomy was performed for small round cell lymphosarcoma. Radiotherapy was employed on three occasions. Eight years following surgery the blood picture revealed lymphatic leukemia with marked lymphocytosis. Although the difficulty in differentiating lymphosarcoma from lymphogenous leukemia by means of biopsy of a lymph node without examination of the bone marrow has been emphasized by Sugarbaker and Craver [10b], Jackson and Parker [11] find that lymphosarcoma and lymphatic leukemia often coexist or the latter may develop in patients with lymphosarcoma whose blood was previously normal. But, they declare, this occurs rarely in adults. Unless leukemia develops the peripheral blood picture of lymphosarcoma is not diagnostic. Whether the administration of x-ray therapy to radio-sensitive lesions is a factor in the development of these cellular metamorphoses or whether cellular



histology *per se* makes differential diagnosis difficult cannot be stated on the basis of the present study. In the four patients in question radiotherapy had been employed.\*

*Results of Surgery.* Nineteen patients died in the immediate postoperative period. Of these, seven had undergone a total gastrectomy, three a laparotomy with only a biopsy specimen being taken, and the remainder a subtotal gastrectomy. Most postoperative deaths occurred before 1943, before the recent advances in anesthesia, blood replacement, postoperative care and use of antibiotics. Five patients died as a result of peritonitis, two from dehiscence of the wound and three from shock or failure to recover from the anesthesia. Three patients died of hemorrhage and five from cardiovascular complications; one patient succumbed to pneumonia.

Twenty-seven patients died within a five-year period; ten of these lived one year or less, seven more than one year but less than two years, five more than two years but less than three, four more than three years but less than four and one more than four years but less than five. Four patients died five or more years following surgery of recurrent disease, i.e., five, six, seven and fourteen years, respectively. Two patients are alive but with recurrent disease more than five and twelve years, respectively, after surgery, the latter following a subtotal gastrectomy for lymphosarcoma and the former following a thoracotomy, biopsy of a cardiac lesion and roentgen therapy.

The brief case histories of several of these patients follow:

A forty-nine year old woman, with a one-year history of abdominal pain died five years following surgery. An intragastric antral mass was found at operation but the lesion was not ulcerated. A subtotal gastrectomy was performed; no lymph node spread was evident. X-ray therapy was not administered. Autopsy at another institution revealed diffuse abdominal spread.

The patient, who lived six years following a subtotal gastrectomy, was a sixty year old man with a

three-year history of abdominal pain. Anacidity was present. A huge ulcerating tumor involving all coats of the stomach and adherent to the pancreas was found. Radiation therapy was administered. The patient died with ascites and recurrent lymphosarcoma.

The patient, who died seven years following surgery, was a fifty-five year old woman with a chief complaint of epigastric pain of three months' duration. She had suffered melena during the six weeks prior to admission. A large flat ulcerated lesion occupying the entire greater curvature was found at surgery. Gastric lymph nodes revealed lymphosarcoma. Subtotal gastrectomy was performed but no x-ray therapy was administered postoperatively. She was readmitted three years later for cervical lymphadenopathy found to be due to lymphosarcoma on histologic examination. X-ray therapy was instituted at this time with some improvement. She died of the disease four years later.

An unusual case is that of a forty-five year old woman with an eight-month history of abdominal distress and weight loss, and a three-month history of dysphagia. A barium meal examination revealed marked obstruction at the cardia and thickened gastric rugae. Esophagoscopy disclosed a dilated esophagus and some leukoplakia. A tentative diagnosis of cardiospasm was made. However, the patient did not improve following dilatation and in view of the roentgenographic findings she underwent exploratory laparotomy. At operation the esophagus and the stomach appeared normal to palpation but the viscus was opened and a biopsy specimen of thickened gastric mucosa at the cardia was removed. Several succulent enlarged lymph nodes were also removed. A jejunostomy for feeding purposes was performed. The histologic report of the mucosal biopsy specimen was small round cell lymphosarcoma. The lymph glands were normal. Following radiotherapy the patient was well for seven years. At that time an abdominal mass presented and a barium meal examination revealed nodular masses in the cardia. Radiotherapy was repeated, and again two years later. She finally died of lymphosarcoma more than fourteen years following the onset of the disease.

Finally, a forty-eight year old woman presented in 1945 with a three-year history of ulcer-type abdominal pain, anorexia and a recent episode of hematemesis. Physical examination revealed tenderness in the left lower quadrant and an intra-abdominal mass. Laboratory examination disclosed a marked hypochromic anemia and no free hydrochloric acid. A barium meal revealed giant rugae and gastroscopy a friable nodular mucosa suggesting carcinoma. A subtotal gastrectomy was performed. Inspection of the stomach revealed diffusely thickened rugae over the antrum and pylorus, with flat, grayish plaques on the surface of the mucosa. Histologic examination disclosed that

\* Some pathologists do not believe that the fundamental histology of a lesion is changed by time or roentgen therapy. The differences in diagnosis are due to a failure on the part of the pathologist to interpret the cell type accurately. These characteristics are more difficult to interpret following x-ray therapy. It is usually necessary to take the pathologic specimen for study from the more central area of the tumor when roentgen therapy has been administered.

only the mucosa and the submucosa were involved. The patient received several courses of radiotherapy but continued to complain of dumping and abdominal pain postprandially. Seven years later a gallbladder series performed because of occasional attacks of distress in the right upper quadrant revealed cholelithiasis. In 1955 anorexia, a palpable spleen and a leukemoid blood picture developed. A short course of x-ray therapy improved the leukemic dyscrasia but the patient complained continuously of abdominal pain and weakness. Repeated barium meal examinations have not revealed any evidence of recurrent disease in the gastrointestinal tract.

Nine patients are alive more than one year but less than five years following diagnosis; four more than one year, two more than two years, one more than three years and two more than four years. Eleven patients are alive and apparently well more than five years following a diagnosis of lymphosarcoma; two more than five years, two more than seven years, one more than nine years, two more than ten years, one more than fourteen years, one more than fifteen years, one more than seventeen years and one more than eighteen years. Four additional patients who are dead of other causes may be presumed to have been cured of their lymphosarcoma. One died of portal cirrhosis fifteen years following the original diagnosis of lymphosarcoma, one seven years later of myocardial infarction, one four years later of cardiac failure and one three years later of coronary artery disease. Postmortem examination in the last two patients failed to reveal any evidence of lymphosarcoma.

*Roentgen Therapy.* Seventeen patients who survived the immediate postoperative period lived less than two years. All of these received roentgen therapy although two did not have apparent lymph node involvement. Ten patients lived more than two years but less than five. Evidence of lymph node metastases was present in seven of these patients. Seven of these received roentgen therapy. In addition, two received nitrogen mustard and one, P<sup>32</sup>. ACTH was employed in four without benefit although the dosage in the light of present experience appears inadequate.

Four patients lived more than five years but are dead of recurrent lymphosarcoma. X-ray therapy was administered to all of these patients. One of them had no involvement of adjacent lymph nodes. One patient is alive more than fourteen years following the original diagnosis but with evidence of recurrent disease. This pa-

tient has received three courses of roentgen therapy. Another patient is alive five years following his diagnosis but with recurrent disease. Fifteen patients were alive and apparently well more than five years after their disease or died of other causes with no evidence of their primary ailment. Radiotherapy was administered to twelve of these patients. Three patients received no radiotherapy; two of these showed involvement of adjacent lymph nodes and in one there was no report of lymph node pathology. The latter (No. 57) is the longest surviving patient in this series; i.e., eighteen years. Another patient in this group without radiotherapy is alive and well fifteen years following surgery. One patient (No. 50) died fifteen years following a subtotal gastrectomy for lymphosarcoma after receiving two courses of roentgen therapy during the first five years of her postoperative period. The last course eleven years before death was directed to a mass in the lower right quadrant that disappeared following therapy. Death was due to portal cirrhosis. Necropsy revealed no evidence of lymphosarcoma. One patient (No. 64) is alive ten years following a subtotal gastrectomy and duodenectomy for disease involving the antrum and duodenum. This patient received roentgen therapy. One patient (No. 22) with reticulum cell sarcoma is alive and apparently well five years following surgery and roentgen therapy; another (No. 23) died of a coronary occlusion four years following a subtotal gastrectomy and radiotherapy. No evidence of reticulum cell lymphosarcoma was found at necropsy.

*Significance of Lymph Node Disease.* Involvement of adjacent lymph nodes was recorded in fifty patients; a negative report was rendered in twenty-one patients and in four there was no mention of the presence or absence of disease of the lymph nodes. Nineteen patients in this series died postoperatively, ten with positive and five with negative reports; twenty-four patients with confirmed lymph node involvement died within a five year period as compared with five who died with negative reports. Five are alive and apparently well from one to five years in the group with established lymph node involvement and three in the negative group. Three patients with lymph node involvement lived more than five years but died of recurrent disease, the longest after a fourteen-year period. One patient with a negative lymph node report is alive more than five years following surgery but with evidence of recurrent disease. On the other hand, seven

TABLE VII  
LIVING MORE THAN FIVE YEARS, OR DEAD WITHOUT EVIDENCE OF RECURRENT DISEASE

Case No.	Age (yr.)	Sex	History	Symptoms	Abdominal Mass	Free HCl	Location	Surgery*	Pathology†	Associated Pathology	Lymph Node Involvement	Radiotherapy	Follow-up	Cured
2	52	F	8 mo.	Anorexia, shoulder pain	+	+	Cardia, esophagus	Gastrotomy	LSA-polypoid	Pneumonia	?	+	3 yr.; died	?
22	43	M	3 mo.	Dyspepsia, nausea, relieved by food	+	+	Antrum	STG	LSA-saucer	Coronary disease	+	+	7 yr.; died	..
23	59	M	4 yr.	Epigastric distress, nausea and vomiting	+	-	Antrum	STG	RCS-annular	Cardiac	0	+	4 yr.; died	?
49	53	F	6 mo.	Pain, nausea, weakness	+	+	Corpus	STG	LSA-saucer	Portal cirrhosis	+	+	15 yr.; died	..
53	56	M	3 mo.	Vomiting, weight loss	+	+	Pylorus	STG	LSA-saucer	.....	+	0	15 yr.	..
54	74	F	6 mo.	Abdominal pain, anorexia, weight loss	+	0	Pylorus	STG	LSA-saucer	Pelvic tumor (radium) 8 yr. before	0	0	9 yr.	..
55	36	M	4 mo.	Pain, vomiting, weight loss	-	+	Pylorus, antrum	STG	LSA-multiple ulcers	Hyperthyroidism, cirrhosis, marginal ulcer	+	+	17 yr.	..
56	67	M	2 mo.	Pain, vomiting, angina	-	+	Antrum	STG	RCS-saucer	Cardiac	+	+	5 yr.	..
57	54	M	1 mo.	Abdominal pain	-	+	Pylorus, antrum	STG	LSA-localized nodules	Cardiac	?	0	18 yr.	..
58	68	M	1 yr.	Abdominal pain	-	+	Corpus	STG	LSA-polypoid	Kaposi sarcoma 5 yr. previously, radiotherapy	0	+	5 yr.	..
61	42	M	3 mo.	Dyspepsia, hematemesis	-	0	Fundus	STG	LSA-submucosa	.....	0	+	14 yr.	..
62	45	F	2 mo.	Ulcer-type pain, vomiting	+	0	Corpus	STG	LSA-mucosa, submucosa	.....	+	+	7 yr.	..
64	65	F	1 mo.	Weakness, pallor	+	0	Pylorus, duodenum	STG + Duod.	LSA-nodular	Hypertension	0	+	10 yr.	..
65	38	M	7 mo.	Ulcer-type pain, dysphagia, vomiting	+	+	Antrum	STG	LSA-polypoid	.....	+	+	7 yr.	..
67	63	M	2 mo.	Ulcer-like pain	-	+	Diffuse	TG	LSA-combined	Duodenal ulcer, myocardial disease	0	+	10 yr.	..

\*STG = subtotal gastrectomy. TG = total gastrectomy.

†LSA = small round cell lymphosarcoma. RCS = reticulum cell sarcoma.



patients are apparently cured of their disease despite definite lymph node involvement while six patients with no evidence of lymph node disease also are apparently cured. In two patients in this group no report of lymph node involvement was available. A comparison of the life expectancy of those patients with positive evidence of lymph node disease and those without lymph node involvement shows an improved prognosis in the latter. (This limited analysis does not take into consideration whether or not roentgen therapy was employed.) The mortality in patients with recognized lymph node involvement was 50 per cent higher than in those with negative reports in the group that lived less than five years. Despite an over-all ratio of 2.5:1 of positive versus negative lymph node reports, the ratio of cure was less than 1.6:1. It is interesting to note that in two patients in whom recurrences occurred five and eight years following surgery there was no demonstrable lymph node involvement at the time of the primary operative intervention.

*Prognosis.* The survival data make clear that a patient who has lived five years following the original diagnosis of lymphosarcoma is not assured of cure. In this respect lymphosarcoma does not conform to the usual standards of cure that are employed for adenocarcinoma. Thus far in this series recurrences of lymphosarcoma have occurred in six patients five years or more after the original diagnosis was made. The longest free interval occurred in a patient in whom recurrence developed nine years following a subtotal gastrectomy for lymphosarcoma. It may be more practical and much safer (Stout) to regard a ten-year interval as the minimal follow-up period in the evaluation of therapy in gastrointestinal lymphosarcoma. (Recently we have seen recurrent disease twenty-five years following a diagnosis of lymphosarcoma of the cervical lymph nodes.) The use of the word "cure" is therefore employed in this study with proper reservations.

In respect to the prognosis of gastric lymphosarcoma with or without lymph node spread and with or without roentgen therapy, the following facts are significant. No patient in the group of twelve who did not receive definitive surgical therapy with resection of the lesion either because of extensive disease or because the diagnosis was not apparent grossly, is cured or alive without evidence of recurrence more than five years after a diagnosis was made. One patient died of pneumonia three years following a

gastrotomy with biopsy of a lesion at the cardia; postmortem examination failed to reveal any evidence of lymphosarcoma. Although this case is included in the "cured" group the relatively brief follow-up raises sufficient doubt to question the therapeutic result. Two others presented recurrent disease after five years, one having died while the other received another course of roentgen therapy. In this regard, however, it is important to note that two patients who received roentgen therapy at least four years following surgery to abdominal masses that were interpreted as recurrent disease made an excellent recovery; one died of portal cirrhosis eleven years later and the other is alive and apparently well three years later. It is also significant that of the twelve patients in whom a total gastrectomy was performed only one (No. 67) is alive more than five years. The remarkable feature of this case is that despite extensive involvement of the stomach the lesion was resectable, the lymph nodes were uninvolved, and an excellent result was obtained. The theory of the multicentric origin of lymphosarcoma must stand on better evidence than this. Yet, other patients with smaller or more localized lesions and without lymph node involvement were less fortunate.

Despite the apparent lack of correlation between the type and degree of pathologic involvement and prognosis, several features deserve comment. Only one of the fifteen patients who presented diffuse disease of the stomach is cured or alive and free of recurrent disease after five years. Another patient presenting diffuse disease involving only the mucosa and submucosa underwent a subtotal gastrectomy. Following several courses of roentgen therapy she appeared improved but recurrence developed eight years later. None of the eleven patients who presented with perforation or a history of perforation in the stomach or duodenum are alive regardless of the method of therapy employed. Of the six patients who had involvement of the duodenum as well as of the stomach only one is alive, and of the three with esophageal involvement none is alive.

Involvement of the mucosa and submucosa alone, without infiltration of the muscularis, may influence the survival rate but the number is too small to be statistically significant. Eight patients presented such localized involvement of the mucosa and submucosa; of these two are alive and apparently cured following surgery and roentgen therapy. One other lived five years but died of recurrent disease.

In this disease, as in carcinoma, the size of the primary lesion bears no relation to the presence of lymphosarcoma in adjacent lymph nodes. In one patient in whom only submucosal infiltration with "cobblestoning" and thickened folds was present, lymph node evidences of malignant dissemination were found. In others with large ulcerated tumors examination of the adjacent nodes failed to reveal any evidence of tumor tissue. However, patients with involved lymph nodes in the resected specimen do not apparently present the poor prognosis that is usually associated with carcinoma.

Fifteen patients in this series were cured or are living five years or more following surgery, with no apparent evidence of recurrent lymphosarcoma. This group consists of ten males and five females; the youngest patient is thirty-six years of age and the oldest seventy-four. The average age is three years younger and the average history is 2.6 months shorter than in the series as a whole. The belief that the onset of lymphosarcoma in the younger patient has a much poorer prognosis cannot be substantiated by the present study of gastric lymphosarcoma. The longest follow-up is in a patient fifty-four years of age at the time of diagnosis who is alive eighteen years after a subtotal gastrectomy. There is no significant symptom complex to distinguish this surviving group. The presence of free hydrochloric acid in the gastric contents does not appear to influence the prognosis. In seven patients an abdominal mass was present. The diagnosis in thirteen of these patients in this series was small round cell lymphosarcoma and in two, reticulum cell lymphosarcoma. The incidence of mucosal and submucosal involvement alone was less than in the entire series, 1:7 as opposed to 1:10. Involvement of the adjacent lymph nodes was present in seven of the fifteen patients in this series, which is significantly less than in the entire group. The number of patients who received roentgen therapy compared to those who did not are in the proportion of 12:3. The administration of radiotherapy may not be an absolute condition for survival if adequate surgery has been performed. On the other hand, radiotherapy alone in the occasional case may be adequate. Nevertheless, the optimum procedure appears to be resection of the lesion followed by radiotherapy. The best prognosis is offered to those patients in whom the neoplasm presents as a localized lesion, ulcerated or not.

*Diagnosis.* The diagnosis of primary lympho-

sarcoma of the stomach may be suspected clinically, and may be suggested radiologically or gastroscopically, but can be established only pathologically. There are no clinical features which characterize lymphosarcoma. The age of the patient appears to be of little aid. In our experience neoplastic gastric disease in the age group between twenty and forty years is more often carcinoma although some reports give the average age of lymphosarcoma at least one decade younger than carcinoma [1,13]. The symptomatology presents no particular distinguishing features. Splenomegaly is often considered a pathognomonic feature; it may be in the far advanced case but in this series it was noted only in eight patients. Normal values for free hydrochloric acid in a patient with a relatively large lesion of the stomach, with or without ulceration, may be a clue to the diagnosis.

Even during laparotomy a diagnosis apparently cannot be made in those patients in whom the infiltration is diffuse and obvious masses are not present. Three patients with perforated lesions, two gastric and one duodenal, were operated on, and simple closure was performed. Apparently, the true character of the lesion was not recognized because the surgical diagnosis was perforated peptic ulcer. There were four instances in which palpation of the stomach by the surgeon did not disclose any significant abnormality. In addition, one patient was operated on following an x-ray diagnosis of gastric ulcer. The surgeon could find no evidence of any disease and the abdomen was closed forthwith. One year later she was found to have definite x-ray and surgical evidence of lymphosarcoma. Obviously, therefore, in the early stages of lymphosarcoma restricted to submucosal involvement, in which mucosal plaques have not yet made their appearance, biopsy offers the only positive identification although x-ray films and gastroscopy may suggest the diagnosis.

In two of the four cases mentioned, a lesion was suspected roentgenologically because of thickened folds, and gastroscopically because of a peculiar discoloration of the mucosa. Nevertheless, accurate roentgen diagnosis still awaits crystallization of more specific criteria. The comparative lack of fibroblastic reaction in lymphosarcoma of the gastrointestinal tract undoubtedly accounts for the relatively good distensibility of the gastric contours, the high incidence of perforation in those areas involved by disease, and the frequency with which a diagno-



sis of gastric ulcer is made. A polypoid lesion may produce a roentgenologic appearance of a benign gastric ulcer or leiomyoma since the ulcerated area extends beyond the gastric wall. When multiple ulcerations are present the diagnosis has been confused with multiple gastric ulcers. On the other hand, a diagnosis of lymphosarcoma is presumptive when the lesion involves the duodenum. The characteristic of carcinoma of the stomach to halt abruptly at the pylorus is well known. The presence of a bizarre gastric pattern also may suggest a diagnosis of lymphosarcoma. Although some of the gastroscopic diagnostic criteria appear adequate [14], in this series a definitive diagnosis was made only in six patients.

*Reticulum Cell Lymphosarcoma.* Reticulum cell lymphosarcoma was encountered eleven times in this series, and although this represents only 15 per cent of the group some features appear significant when compared with the findings in the entire series. The average age of onset was approximately the same as for the entire series, fifty-eight years, but the average history was shorter, 5.8 months as compared with a nine-month period for the series as a whole. There were six males and five females, a more equal distribution of the sexes. The characteristics of the gross lesion and its relative incidence in this group did not differ from that observed in the series as a whole; nor was the anatomical location of the lesion any different than in the entire series. However, in the patients with reticulum cell lymphosarcoma there were four gastric perforations with abscess formation as against eleven instances in the entire series. This reveals an incidence of perforation almost three times as high for reticulum cell lymphosarcoma as for small round cell lymphosarcoma. In its tendency to spread beyond the confines of the stomach, reticulum cell lymphosarcoma again behaves more aggressively than does small round cell lymphosarcoma. In this group of eleven patients invasion of the duodenum was encountered in two, of the esophagus in one. Surgery and roentgen therapy appear slightly less effective in reticulum cell lymphosarcoma than in small round cell involvement. In this group of eleven cases seven patients are dead. There is one apparent five year cure (Case No. 56) and one patient died of myocardial disease four years following surgery (Case No. 23). Postmortem examination failed to reveal any evidence of lymphosarcoma in this patient,

and this may be considered a "cured" case. Another patient lived four years following surgery but died of recurrent disease. Two patients are alive and apparently well four and three years, respectively, following surgery. Although reticulum cell lymphosarcoma is generally considered to have a uniformly unfavorable prognosis, particularly when compared with small round cell lymphosarcoma, that impression cannot be wholly substantiated by the present series [15].\*

The following case histories are illustrative of patients who appear to have been "cured":

CASE 49. A fifty-three year old white woman presented with a chief complaint of abdominal pain and nausea of six weeks' duration. The pain was epigastric in origin, unremitting, with occasional radiation to the back. More recently it became diffuse, involving the entire abdomen, and was accompanied by nausea. During the previous six months she suffered from weakness and dizziness. On physical examination a tender globular mass was palpated in the right hypochondrium. This was interpreted as an enlarged gallbladder. Laboratory examination revealed 90 per cent hemoglobin, a normal white blood cell count and differential, and a stool guaiac of 2 plus. Barium meal examination revealed what appeared to be a normal stomach. Slight thickening of the mucosal folds in the antrum was considered to be within normal limits. There was no gastric retention. The duodenal bulb filled adequately but was flattened on the lesser curvature side. At laparotomy a small firm mass was palpated on the lesser curvature aspect of the pylorus of the stomach. A subtotal gastrectomy was performed. Pathologic examination revealed that near the re-entrant angle and saddling the lesser curvature aspect of the stomach was a crater-like ulcer 2.5 cm. in diameter surrounded by very firm hard rolled-in edges. The surrounding mucosa was thickened and radiated to the margins of the ulcer. The ulceration extended through the gastric wall to involve the fat on the lesser curvature; on the posterior wall, the lesion extended to the serosa.

The pathologic diagnosis was lymphosarcoma with ulceration and lymph node involvement. The patient did well and was not seen until five years later when a mass in the right periumbilical region was palpated. The spleen was not felt. The mass was considered to be recurrent disease and was treated by radiotherapy.

\* Difficulties in differential diagnosis between reticulum cell sarcoma and anaplastic carcinoma are well known. This is especially true, in the early development of the lesion. It is possible that the extremely poor prognosis recorded previously in reticulum cell sarcoma may be due to this difficulty, i.e., some of these lesions may have been anaplastic carcinoma rather than reticulum cell sarcoma.



The mass disappeared. One year later she was readmitted for a lower right nodular mass which again disappeared following another course of roentgen therapy. Two years later the patient was seen by a private physician because of edema of the legs, enlargement of the liver and portal hypertension. Symptoms of hepatic disease became progressively more prominent and jaundice and ascites developed. In 1950, fifteen years after the original diagnosis of lymphosarcoma, she died at another institution. Necropsy revealed advanced Laennec's cirrhosis. No evidence of malignancy was found.

**CASE 55.** A thirty-six year old white man was admitted with a chief complaint of pain in the left hypochondrium of four months' duration. The pain appeared to be aggravated by ingestion of food at which time it radiated around to the back. During the four weeks prior to admission there were intermittent episodes of vomiting, usually after meals. He had suffered a recent weight loss of 15 pounds.

Physical examination revealed tenderness in the right upper quadrant. Laboratory examination disclosed a normal erythrocyte sedimentation rate, and a white blood cell count of 7,900 per cu. mm. with an 11 per cent monocytosis. Following an Ewald test meal, 40 units of free hydrochloric acid were found. Barium meal examination revealed an irregular filling defect with crater formation in the antrum and pylorus of the stomach, suggestive of an ulcerating carcinoma of the stomach. At laparotomy a large mass encircling the lumen of the stomach was found and a subtotal gastrectomy was performed. The resected specimen revealed a mass 10 by 18 by 2 cm. at the distal end of the stomach. The lesion ended sharply at the duodenum. The mass presented three irregular ulcerations measuring 1 to 3 cm. in diameter. (Fig. 3.) The tumor tissue was quite elastic. It involved all the layers of the stomach to form extragastric masses on the lesser curvature. The pathologic diagnosis was lymphosarcoma with involvement of the subjacent lymph nodes. X-ray therapy was employed.

Follow-up examinations at frequent intervals gave no indication of recurrent disease. Ten years following the surgical intervention the patient was admitted to another institution because of jaundice. At this time a diagnosis of portal cirrhosis was made. Six months later he was readmitted with thyrotoxicosis. He was treated with radioactive iodine, with improvement. Thirteen years following the original diagnosis he was readmitted because of abdominal pain temporarily relieved by food. Heartburn and sour regurgitation were other complaints. Gastric analysis disclosed 30 units of free hydrochloric acid. Barium meal examination at this time revealed a gastrojejunal ulcer. A diagnosis of stomal peptic ulcer was made; there appeared to be no other involvement of the gastrointestinal tract. Medical therapy was followed by relief of symptoms. Two years later he was seen be-

cause of bright red rectal bleeding of one month duration. The blood was seen to come from several internal hemorrhoids. Treatment was conservative. At the present time there is no evidence of recurrence of his lymphomatous disease.

**CASE 56.** A sixty-seven year old white man was admitted with a history of indigestion and abdominal pain of ten weeks' duration. The abdominal pain was generalized and occurred usually after meals. It was relieved by induced vomiting. The indigestion consisted of abdominal distress, bloating, belching and discomfort. In addition there were symptoms of angina and orthopnea. This patient had incurred a weight loss of 32 pounds during a four-month period.

Physical examination revealed some sclerosis of the retinal vessels. Examination of the abdomen was within normal limits. Laboratory examination revealed guaiac-positive stools, 30 clinical units of free hydrochloric acid following the administration of histamine, and a normal blood count. Barium meal examination (performed elsewhere) disclosed a filling defect in the antrum of the stomach; the presumptive diagnosis was carcinoma. The patient was digitalized and a subtotal gastrectomy with resection of a portion of the omentum performed for a mass involving the distal third of the stomach. The tumor measured 6 by 7 cm. and presented on the lesser curvature and posterior wall of the antrum. On the mucosal surface, the central portion of the tumor was necrosed and covered by an adherent grayish green exudate; the margins were overhanging and the edges were firm and indurated but the adjacent mucosa was freely movable. The serosa was fixed and presented several pale green nodules. The cut surface presented a homogenous tannish white appearance with extension of the tumor tissue as thin white streaks into the muscularis. The pathologic diagnosis was reticulum cell lymphosarcoma with evidence of lymph node spread. X-ray therapy was employed. Postoperatively, some obstruction developed, apparently at the stoma, with moderate gastric retention. Following intubation this was promptly relieved.

The patient was last observed five and a half years later with arteriosclerotic heart disease but with no evidence of recurrent lymphosarcoma.

**CASE 57.** A fifty-four year old white man presented with a history of epigastric pain of four weeks' duration. The pain was non-radiating, apparently unrelated to food and was not associated with vomiting. There was no weight loss. Physical examination was entirely within normal limits. The stool guaiac test was negative. A gastric analysis revealed 68 units of free hydrochloric acid following an Ewald test meal. A barium meal examination was interpreted as carcinoma of the pylorus. At surgery a firm nodular mass 6 cm. from the pylorus was found on the lesser curvature aspect of the stomach. A subtotal gastrectomy including the proximal duodenum was per-

formed. The tumor mass consisted of five distinct ulcerated nodular masses the largest of which was 3 cm. in diameter and limited to the mucosa and submucosa. Although on gross examination the duodenum appeared involved, more careful examination did not confirm this. The remainder of the stomach was entirely normal. The pathologic diagnosis was small round cell lymphosarcoma. No roentgen therapy was instituted.

This patient has been seen on several occasions during which time barium meal studies were performed. He is alive nineteen years following surgery, with symptoms of cardiac failure but with no evidence of recurring disease.

**CASE 62.** A white woman, forty-five years of age, complained of epigastric pain of two months' duration. The pain occurred after meals, would radiate to the back and was relieved by the ingestion of food. Four years prior to the present illness she had suffered intermittent attacks of painless vomiting that had persisted over an eighteen month period.

On physical examination a large, hard mass was palpated in the upper part of the abdomen. Barium meal examination revealed a prepyloric lesion on the greater curvature aspect of the stomach. The diagnosis was carcinoma. At surgery a large tumor was found on the greater curvature of the stomach at the prepyloric area, with questionable metastases to the liver. A subtotal gastrectomy was performed. Pathologic examination disclosed a soft 6 cm. mass involving the anterior wall and greater curvature aspect of the pyloric end of the stomach. The mass showed central necrosis and ulceration; the margins of the ulcer were markedly indurated. Hemorrhage into the body of the mass had occurred. Section through the tumor showed no involvement of the muscular coat. The pathologic diagnosis was lymphosarcoma with lymph node involvement.

At present the patient suffers from a moderately severe dumping syndrome seven years after definitive therapy.

**CASE 64.** A sixty-five year old white woman presented with a two-week history of abdominal cramps and tarry stools for twenty-four hours. The abdominal cramps were generalized and appeared unrelated to food intake. A large tarry stool was passed shortly prior to admission, followed by fainting, severe weakness and sweating. During the previous four years she had lost 10 pounds in weight. For ten years she had been treated for essential hypertension.

Physical examination revealed a poorly nourished pale woman with a blood pressure of 155/65 mm. Hg. A large mass presented in the lower right quadrant. Laboratory examination disclosed 1.9 million red blood cells per cu. mm., hematocrit of 15 per cent and a 4-plus guaiac reaction in the stool. The  $\text{CO}_2$  combining power was 29 vol. per cent. The electrocardiogram revealed myocardial disease.

Following correction of hematologic and electrolyte deficiencies, the patient was operated on. A large antral mass that appeared to involve the duodenum was found. Subtotal gastrectomy and proximal duodenectomy was performed, as well as an enteroenterostomy. Pathologic examination revealed a firm white nodular bulge on the serosal surface involving the pylorus and the duodenum, and measuring 2.5 cm. On the mucosal side there was a firm submucous tumor; no ulceration was present but the mucosa was fixed and thickened. The neoplasm was beginning to invade the muscularis. The diagnosis was small cell lymphosarcoma involving the stomach and duodenum. X-ray therapy was instituted. Eight years following surgery the patient complained of occasional heartburn but routine roentgen and clinical studies revealed no evidence of recurrent disease.

**CASE 67.** A sixty-three year old man was admitted to the hospital with a chief complaint of epigastric pain of two months' duration. The pain was knife-like, radiated to the back, and occurred two hours following meals. The pain was relieved by food but not by antacids. During the past month he could tolerate small meals only. During the present illness he lost 4 pounds in weight. His first admission to Mount Sinai Hospital had been nine years previously for a bleeding duodenal ulcer. The duodenal ulcer had originally been diagnosed thirty years previously. An old myocardial infarction due to arteriosclerotic heart disease was noted.

Physical examination revealed no significant heart disease. The blood pressure was 160/90 mm. Hg. The liver edge was firm, smooth and palpable 1.6 cm. below the right costal margin. The hemoglobin was 13.5 gm. per cent. The stool was positive for occult blood. Following an Ewald test meal 34 mEq./L. of free hydrochloric acid was present in the gastric aspirate. Gastroscopic examination revealed a 5 cm. ulcerated tumor with thick everted margins on the greater curvature aspect of the posterior wall of the stomach, above the angularis. Another lesion approximately 5 cm. in size and similar in appearance was seen on the lesser curvature aspect of the stomach. The rugal folds were prominent throughout. The gastroscopic diagnosis was carcinoma. Barium meal examination revealed marked mucosal distortion. A rigid segment of approximately 10 cm. with no evidence of peristalsis was visualized on the greater curvature aspect opposite the angularis. On compression a 1 cm. crater was identified, the folds were non-radiating, and a halo surrounded the crater. The antrum appeared normal. The roentgenographic impression was carcinoma or leiomyosarcoma. At operation the entire stomach appeared involved and a total gastrectomy was performed. A supplemental feeding jejunostomy was provided. Pathologic examination revealed marked thickening of the entire stomach. Two walnut-sized, firm, white nodular

masses projected from the serosa. The mucosa along the greater curvature was replaced by an irregular, firm, flat mass with four large saucer-like ulcerations. The edges of the ulcers were indurated and flat, the base was yellowish white. The rugae were thick, high and firm between the lesions. The lesion infiltrated the stomach from an area 6 cm. from the pylorus to the esophagus. Despite the extent of the lesion no lymph node involvement was found. Roentgen therapy was administered.

Two years later this patient had gained 10 pounds and offered no complaints. Five years later he suffered an acute attack of gallbladder colic. Roentgenographic studies revealed cholelithiasis and a cholecystectomy was performed. The opportunity afforded to explore the abdomen yielded no evidence of recurrent disease. Convalescence was uneventful. When last heard from, ten years after his operation, the patient was in good health.

#### SUMMARY

1. An analysis is made of seventy-five patients with primary lymphosarcoma of the stomach, followed up for periods ranging from one month to twenty years. Sixty-four had histologically verified small round cell lymphosarcoma; eleven had reticulum cell lymphosarcoma.

2. No characteristic clinical or laboratory findings could be discerned. The finding of normal or high values of free hydrochloric acid in the presence of large, evidently malignant lesions of the stomach may suggest lymphosarcoma.

3. Perforation occurred in eleven cases (14 per cent). Cases with this complication offer the poorest prognosis.

4. The pathologic appearance of lymphosarcoma is multiform-infiltrative, ulcerative, nodular, polypoid and combined lesions. The ulcerative form, occurring as a shallow, localized ulceration, was present in 42 per cent of the cases, the most frequent type encountered.

5. Involvement of the duodenum was noted in six patients, of the esophagus in three.

6. Fifteen patients (20 per cent) are alive more than five years after the diagnosis was established, or died after five years of other causes, with no evidence of recurrent lymphosarcoma. Seven patients are alive and presumed to be cured more than ten years after surgery, with no evidence of recurrence. Because recurrences appeared as late as nine years following treatment, the prognosis in gastric lymphosarcoma must be guarded.

7. The most effective program of treatment appears to be definitive surgery followed by

radiation. Rarely, a patient may be cured by roentgen therapy alone, more frequently by adequate surgery alone.

8. The prognosis in patients with gastric lymphosarcoma and lymph node involvement appears to be more favorable than with carcinoma of the stomach with comparable spread.

*Acknowledgment:* I am indebted to Drs. John Garlock, Ralph Colp, Leon Ginzburg and Burrill Crohn for permission to use their extensive case records and to Dr. Richard Marshak who stimulated our interest in this study. Figures 2, 3 and 4 were made available through the courtesy of Dr. Sadao Otani, Pathologist, The Mount Sinai Hospital, New York, New York, and Figure 1 through the courtesy of Dr. V. Gillson, Pathologist, Bergen Pines County Hospital, Paramus, New Jersey. I wish also to express my thanks to the New York City Department of Health and Dr. L. Baumgartner, Commissioner, for their cooperation and help in the follow-up of some patients in this series.

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# Seminar on Connective Tissue

## Remarks on the Present State of the Rheumatoid Arthritis Problem\*

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RECENT studies on rheumatoid arthritis have been concentrated in three fairly well defined areas—the clinical disease, the tissues involved, and the serologic reactions observed. Each area has been intensively investigated in relationship to pathogenesis. An evaluation of the current status of this work might prove illuminating.

*Clinical Studies.* The clinical picture of the disease—its natural history [1,2] and its epidemiology [3]—has received a great deal of attention. These efforts on a clinical plane have been fraught throughout with difficulties imposed by the elusive nature of rheumatoid arthritis. The disease when far advanced is apparent even to the novice but in its early or minimal stage is difficult to recognize and to characterize even for the trained specialist. Diagnostic criteria have been proposed but they have serious limitations [4]. Criteria to appraise the rate and intensity of progression of the disorder have been suggested but these also have defects [5,6]. Individual patients have been noted who violate all the criteria, and the frequency with which these patients are encountered has sorely tried the investigator who has chosen to work with rheumatoid arthritis. All dicta promulgated for assessing (1) the presence of the disease, (2) its severity, and (3) its expected course, can be applied only to rheumatoid arthritis in general and not to the individual patient. And, lastly, functional capacity correlates with severity of disease on a statistical but no more precise basis.

From these studies certain broad generalizations may however be made. Patients are more likely to have sustained disease if the manifestations of the disease are "typical" rather than

"atypical" [7]; "classic" rather than "probable," according to the proposed diagnostic criteria [7]; with a positive serologic reaction for the rheumatoid factor rather than with a negative reaction [2]; men do better than women [7]. In population studies of patients with classic rheumatoid arthritis, i.e., those patients exhibiting positive "factor" reactions and x-ray changes, the sex incidence approaches 1:1, in contrast to the usual clinic population ratio of two to three females to one male. In such total population studies, however, patients with joint signs and symptoms (but not necessarily factor-positive or x-ray-positive) have a similar female preponderance [3].

The hereditary aspects of peripheral joint rheumatoid arthritis are not as clear-cut as they appear to be in the spinal variant, Marie-Strümpell spondylitis [19]. Peripheral joint rheumatoid arthritis occasionally appears in twins, and certain families have been encountered with a high prevalence of the disease. In three studies the rheumatoid factor has been observed more frequently in siblings [3,7,8] but in one the technic used is considered by some to be too sensitive and consequently non-specific [8]. No conclusive pedigree studies on the disease has been carried out to date. Most students of the disease believe that genetic factors probably play a role in peripheral joint rheumatoid arthritis but one which will not be easy to document.

Assessment of therapy has been particularly difficult. An attempt is currently being made in this country to determine the possibility of employing modern methods of therapeutic evaluation in this disease, with its characteristics of chronicity, unpredictable course, and end-

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points so difficult to appraise. In England similar efforts have been made [9] but reservations have been expressed concerning the validity of these trials [10].

All in all, the relevant clinical studies of rheumatoid arthritis have been tedious and not too rewarding. Although a great mass of data has been accumulated from such studies, we have gained from them but little solid knowledge of the cause of the disease.

*Connective Tissue Studies.* The study of the tissues involved in rheumatoid arthritis, in relation to etiologic mechanisms, has had a long and honorable history. The histopathology of rheumatoid arthritis may be called nondescript. In patients with unequivocal disease the lesions are similar to scar tissue with an element of granulomatous inflammation. In early disease, the specificity of the lesion observed may be questioned. The rheumatoid nodule is considered to be the most characteristic visible lesion but the distinction between the nodule of rheumatoid arthritis and that of rheumatic fever remains a matter of dispute, and the fact that rheumatoid nodules appear in patients with unequivocal systemic lupus erythematosus [17] tends to lessen the specificity of this so-called specific lesion. It has been proposed on the basis of limited direct evidence, as well as on inferential evidence, that the primary lesion is an arteritis [12,13]. But one or another form of arteritis may be encountered in a myriad of disorders and this view, even if correct, can therefore hardly lead to a closer delineation of our knowledge regarding possible pathogenic mechanisms. It has suggested to some the possibility that the disease may be based on hypersensitivity. This train of thought has been fortified by the lack of conclusive evidence of a microbial etiology, by the sustained nature of the disease in many instances, and by the presence of the rheumatoid factor which, it has been suggested, may play the role of an antibody, particularly an autoantibody.

The disease affects the connective tissue widely and has loosely been called—with some justification since it involves this tissue primarily—a disease of the connective tissue. In the last decade, interest in this aspect of the problem has stimulated and has led to the support of much work in this previously neglected area. A great deal has been learned about the connective tissue, its cells, amorphous ground substances and fibrillar elements [14]. Ignorance even of the

composition of the connective tissue was so monumental when the work began that our understanding of this aspect of normal tissue has completely occupied the attention of many investigators and they have been able to approach the problem of abnormal states only sporadically. It is hoped that in time an understanding of abnormal conditions will logically follow the elegant work which has so far been done on the composition of normal components. Another aspect of the problem, i.e., the plasticity of this tissue, its genesis, differentiation, turnover and the role it plays in vital processes, has been touched upon even in normal states only in the most superficial fashion [15]. Suffice it to say, however, that as of now, little knowledge of the pathogenic mechanisms involved in rheumatoid arthritis has evolved from these truly great additions to our general biologic knowledge.

*The Concept of Autoimmunity in Rheumatoid Arthritis.* The autoimmune aspects of the problem have developed in two separate channels. The first consists of attempts to determine the antigenicity of components of homologous connective tissue. These have been unsuccessful to date, and there is a real possibility that isolation procedures may destroy the potential antigenicity of the substances under investigation.

The second approach involving the concept of autoimmunity has concerned itself with the possible role of the rheumatoid factor as an antibody. Significant advances have been made in our understanding of the reaction in which this material participates [16]. In brief, and in oversimplified terms, the reaction involves the factor residing in the 19S fraction of gamma globulin (which contains other antibodies) and the reactant material with which the factor reacts. This latter substance is, in the precipitating and some agglutinating systems, an aggregate of 7S gamma globulin; in other agglutinating systems it consists of antibody gamma globulin in combination with its specific antigen—as in an immune precipitate. In other words, the factor reacts with gamma globulin antibody only in the physical state in which gamma globulin exists when it is combined with specific antigen. A soluble complex with a sedimentation constant of 22S apparently is formed, consisting of the factor and non-aggregated gamma globulin. The interpretation of the difference in reactant material in precipitating and agglutinating systems on the one hand and the soluble complex on the other is not clear at this time.

The reactant is species-non-specific. Similarities and dissimilarities between the rheumatoid factor and antibodies are frequently reported. Crude attempts made to induce the disease, or to modify it once established, by injecting the factor [17] or the reactant [18] have so far been unsuccessful. Continued study of the role of the rheumatoid factor in the disease is a lead which must be pursued. At the time of this writing, the thought that this reaction may turn out to be of the Wassermann reaction type and lead us no further towards knowledge of pathogenesis is uppermost in many minds.

Thus, in summary, the ideas which have been followed have not, up to now, led to a clear-cut understanding of the pathogenesis of rheumatoid arthritis. The picture, however, is not all black. With the interest which has been aroused by each new development, new investigators have been attracted to the field and it is to be hoped that these new minds will continue to develop fresh approaches. Control of rheumatoid arthritis need not wait upon complete elucidation of the basic mechanisms involved. The demonstration that the process of rheumatic fever was initiated by infection with the Group A hemolytic streptococcus is an example. Antistreptococcal prophylaxis for the prevention of recurrent attacks of rheumatic fever has been eminently successful in curbing that disease, yet we have little more understanding of the subsequent stages of rheumatic fever following the initial streptococcal infection than we have of rheumatoid arthritis. More complete comprehension of the precipitating causes of rheumatoid arthritis, its initiation, and its ability to sustain itself might provide the means whereby the chain of progression of the pathologic process could be broken. As an example of this, there is a clinical oddity which has been recognized for a long time. When the patient with rheumatoid arthritis receives an insult, whether traumatic or infectious (a bout of pneumonia), the symptoms of the disease may abate temporarily. This cannot be satisfactorily explained by an increase in endogenous steroid secretion or other known factors. We have naively considered that the patient with rheumatoid arthritis cannot manage more than one disease at a time but a logical explanation based on knowledge of pathogenicity would obviously be more satisfying.

It is still possible, of course, that further understanding of the clinical picture of the disease, its morbid pathology, the characteristics of the

connective tissue or the role of the rheumatoid factor may help to unravel the problem of the pathogenesis of rheumatoid arthritis. Obviously, each of these areas needs continued study. In addition, new investigators recently brought into this field may develop a fresh approach which would fulfill the wish of those who have given categorical funds for the prevention and cure of rheumatoid arthritis. New concepts require an idea, some prior knowledge, and a dash of serendipity. They cannot be bought, but the development of a fresh concept and its pursuit to a successful conclusion can be helped immensely by such funds.

Success in this framework would consist of ability to break the chain of progression of events which we know as the disease "rheumatoid arthritis." Total success would mean ability to prevent the inception of the disorder, but most of us would be quite happy to be able to modify the disease for the benefit of the patient by measures which, in addition to being reliable and relatively non-toxic, would be based logically on an understanding of pathogenic mechanisms.

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# Case Reports

## Parathyroid and Pancreatic Adenomas\*

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IN 1903 Erdheim [1] reported a case of co-existing tumors of the parathyroid and pituitary glands. Since this initial report forty-five additional cases of multiple endocrine adenomas involving the pancreas, parathyroid and pituitary glands have been published [2-10]. We describe here a case of parathyroid and pancreatic adenomas of particular interest because the patient had presented himself with signs referable to a massive parathyroid tumor eight years before this or any other tumor became symptomatically manifest.

### CASE REPORT

J. C., a forty-four year old white male machine operator, was first referred to another hospital in 1949 because of an intrathoracic mass discovered on routine roentgenographic examination of employees. The patient was asymptomatic and denied previous illness. The family history was non-contributory.

Physical examination revealed an asthenic, middle-aged man who appeared neither acutely ill nor chronically ill. He was noted to be mildly hypertensive (180/100 mm. Hg) and this was an inconstant finding throughout his eight-year follow-up. Percussion of the right posterior chest in the area of the fifth dorsal vertebra revealed a round area of dullness. The remainder of the physical examination was not significant. Urinalysis, blood urea nitrogen and blood sugar were within normal limits; a maximum urea clearance was slightly depressed. The basal metabolic rate was normal, the serum phosphorus was 1.4 mg. per cent, and alkaline phosphatase was 3.1 Bodansky units. The serum calcium was not determined.

A roentgenogram of the chest and fluoroscopy revealed a 4 by 4 cm. mass at the level of the aortic arch to the right of the trachea, with slight displacement of the esophagus to the left. It was non-calcified and non-pulsatile. Physical and fluoroscopic examination showed no evidence of laryngeal or phrenic nerve involvement, nor did the mass appear to communicate with the thyroid gland. A tracer dose of radioactive iodine showed no sign of uptake in the mediastinal mass. Bronchoscopy revealed a smooth,

regular indentation of the right tracheal wall without evidence of endobronchial disease. Biopsy of the left axillary lymph node showed only chronic lymphadenitis.

A trial dose of radiotherapy was administered to determine whether or not the tumor was radio-sensitive. After eight days, during which a total dose of 1,000 r was delivered to each of two ports without signs of regression of the mass, the patient left the hospital against advice and was lost to follow-up for the next four years.

In 1952 the patient was first admitted to Bellevue Hospital following a fall with resultant fracture of the right tibia. He gave a history of a non-productive cough since leaving the other hospital. In addition, three years prior to this admission he had had two brief episodes of passage of dark red urine which cleared spontaneously. Subsequently he noted nocturia without dysuria. Physical examination at this time was unchanged from that reported on admission to the other hospital. A roentgenogram of the chest (Fig. 1) showed persistence of the mediastinal mass without alteration in size, and fluoroscopy confirmed the absence of expansile pulsations. The serum non-protein nitrogen was within normal limits. Urinalysis revealed albuminuria, without formed elements, which persisted throughout his subsequent course.

On the twelfth hospital day the fracture was reduced, with insertion of Kirschner nails. At operation no abnormality of the bones other than fracture was noted. His subsequent course was uneventful, with good return of function in the right leg.

In 1954 he was admitted to the urological service for repair of a right hydrocele which had been present for five years. Physical examination at that time revealed a mild dorsal kyphosis with increase in the anteroposterior chest diameter. Roentgenographic findings were unchanged from those of his previous two admissions. Albuminuria persisted; the serum non-protein nitrogen remained normal. Cystoscopy revealed no prostatic hypertrophy, and the patient underwent a hydrocelectomy with uneventful recovery. During this admission the patient had an intravenous pyelogram which was reported as showing prompt bilateral function and normal contours

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FIG. 1. Chest roentgenogram taken on May 29, 1952, showing mediastinal mass. This is similar in size and configuration to that described in 1949.

but, in retrospect, revealed scattered densities in the lower pole of both kidneys suggestive of nephrocalcinosis and nephrolithiasis. (Fig. 2.) The serum phosphorus was 1.8 mg. per cent, the alkaline phosphatase was 7.6 Bodansky units. The serum calcium was not determined.

Throughout the succeeding three years his cough persisted in association with orthopnea and progressive dyspnea on exertion. He continued to have nocturia three to four times accompanied by increasing symptoms of bladder neck obstruction. On two occasions he noted gross clots of blood in his urine, which subsided spontaneously after two or three days and were not associated with abdominal pain or fever. Following these episodes severe generalized pruritus and anorexia developed, and he had a progressive weight loss amounting to 30 pounds in two months.

During the six to eight months prior to his final admission a penetrating ache in his right shoulder which increased with motion and local pressure impelled him to return to the hospital. X-ray films of the shoulder revealed considerable distortion of the head and proximal portion of the right humerus and widespread osteolytic changes and fracture of the posterior third of the right fourth rib. He was readmitted with a tentative diagnosis of metastatic prostatic carcinoma.

Physical examination revealed cachexia and marked kyphosis. On gross examination of the cornea calcium deposits were evident in the limbal areas which on slit lamp were underlying Bowman's membrane bilaterally. Similar calcifications were noted in both tympanic membranes. The trachea was deviated to the left, and scattered rhonchi and wheezes were heard throughout both lung fields. The heart was enlarged to the left, with evidence of mild left ventricular hypertrophy confirmed on fluoroscopy and electrocardiogram. The patient was orthopneic but there was no venous distention or



FIG. 2. Intravenous pyelogram taken on March 22, 1954. Film was taken three minutes after injection of the dye. Note the scattered densities in the lower pole of both kidneys suggesting nephrocalcinosis.

elevation of arm venous pressure. The bladder was enlarged to percussion but residual urine could not be measured owing to difficulty passing a catheter. Rectal examination showed that the prostate was only mildly enlarged and symmetric except for a 1 by 1 cm. firm nodule lateral to the left lobe. The fingers were referred to as "clubbed" by several examiners, but this finding was subsequently attributed to marked shortening and distortion of the terminal phalangeal tufts.

Laboratory studies revealed a normochromic, normocytic anemia with a normal bone marrow and reticulocyte count. There was a mild leukocytosis. Repeated stool examinations were negative for occult blood. Urinalysis revealed a specific gravity fixed below 1.010 in repeated specimens, persistent proteinuria, and numerous red and white cells without casts. Studies were negative for bile and Bence Jones protein, and urobilinogen excretion was within normal limits. Culture of the urine revealed *Bacillus proteus* and *Escherichia coli* resistant to all antibiotics. The blood urea nitrogen initially was 200 mg. per cent. The standard urea clearance was 1.6 cc. per minute, and the phenolsulfonphthalein excretion was less than 10 per cent in one hour. The serum electrolytes remained normal throughout the course except for an initial phosphorus of 9.8 mg. per cent, which fell to values ranging from 3.0 to 4.7 mg. per cent following institution of therapy with aluminum hydroxide gel. Repeated serum calcium determina-



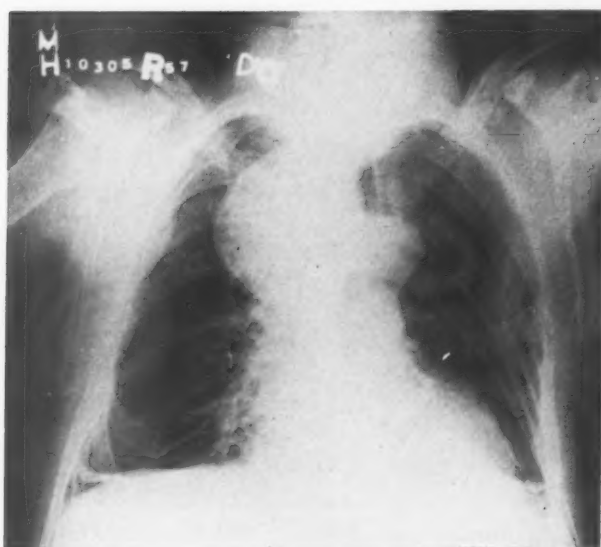


FIG. 3. Roentgenogram of the chest taken on February 25, 1957, again showing the mediastinal mass, unchanged except for the presence of a thin rim of calcification.

tions ranged in value from 10.2 to 10.9 mg. per cent and a single urinary excretion study revealed a total of 42 mg. in twenty-four hours on a high calcium intake. The alkaline phosphatase ranged from 9.3 to 19.0 Bodansky units on serial determinations, with repeated normal serum acid phosphatase. The serum protein was 6.7 gm. per cent with an albumin fraction of 4.2, and a single blood sugar was 94 mg. per cent.

A roentgenogram of the chest (Fig. 3) again revealed the mediastinal mass, unchanged in size or appearance except for the presence of a thin, unbroken rim of calcification. The esophagus was displaced posteriorly. (Fig. 4.) On abdominal films (Fig. 5) a similarly calcified although smaller mass was noted overlying the left iliac crest anterior to the first lumbar spine. Diffuse calcification involved all the medium-sized arteries including those in the abdomen, legs and arms, and patchy areas of calcification surrounded the greater trochanter of the left femur, the lateral margin of the right acetabulum, and were present in the lower pole of both kidneys as previously described. There was widespread cystic degeneration of the bones of the hip, both humeri (Fig. 6), both femora, and the small bones of the hands. (Fig. 7.) Neither the abdominal mass, the widespread calcification nor the cystic bony changes had been present on previous abdominal films. (Fig. 2.) Films of the skull were within normal limits as they had been in 1952, both with respect to the bony vault and the size and shape of the sella turcica.

Catheter drainage was instituted, together with a liberal fluid intake. During this regimen his urinary output remained high, his weight constant, and his blood urea nitrogen fell to 90 to 100 mg. per cent. Following repeated transfusions his hematocrit rose

MAY, 1959



FIG. 4. A left anterior oblique film of the chest with barium swallow taken on February 25, 1957, showing the relationship of the mediastinal mass to the esophagus and aorta.

from 29 per cent to 47 per cent and he remained afebrile while receiving Gantrisin® and tetracycline, without change in hematuria or pyuria. Starting on the twentieth hospital day he manifested a low grade fever. On the thirtieth hospital day his temperature suddenly spiked to 103°F. and was associated with the outbreak of a diffuse petechial eruption principally involving the trunk and proximal extremities, and this was followed immediately by intense sweating, confusion, stupor and hypotension. The patient died despite emergency measures. Blood cultures drawn at this time subsequently revealed *E. coli* in all specimens.

An autopsy was performed two hours after death. Unfortunately, permission for examination of the brain was not granted. On gross examination the principal features were the findings in the neck, mediastinum and abdomen. Behind the thyroid on the right was a single normal parathyroid gland, while on the left both parathyroids were greatly enlarged and nodular, with a long diameter of about 2 cm. each. Subsequent section of these glands revealed multiple circumscribed areas of parathyroid parenchyma in which chief cells predominated. These areas were surrounded by delicate fibrous septums and scattered regions of calcification and hyalinization of stroma.

The mediastinum was deviated to the right by the 7 by 7 by 6 cm. globose mass which on cut section revealed a stout, fibrous capsule containing calcified



FIG. 5. Film of the abdomen taken on March 4, 1957, showing calcified abdominal mass and widespread calcification involving arteries, hip joints and lower pole of both kidneys. Note the sclerosis and cystic degeneration of the visualized bony structures.

deposits surrounding a homogeneous mass of old clotted blood. No connection could be demonstrated between this cyst and the neighboring blood vessels, pericardium, bronchus, trachea or esophagus. Section of the capsule revealed a layer of hyalinized connective tissue surrounded by an irregular zone of



FIG. 7. Film of the hands taken on March 14, 1957, showing advanced osteitis fibrosa cystica. Note the presence of subperiosteal cystic degeneration in some areas, and disappearance of the phalangeal tufts.

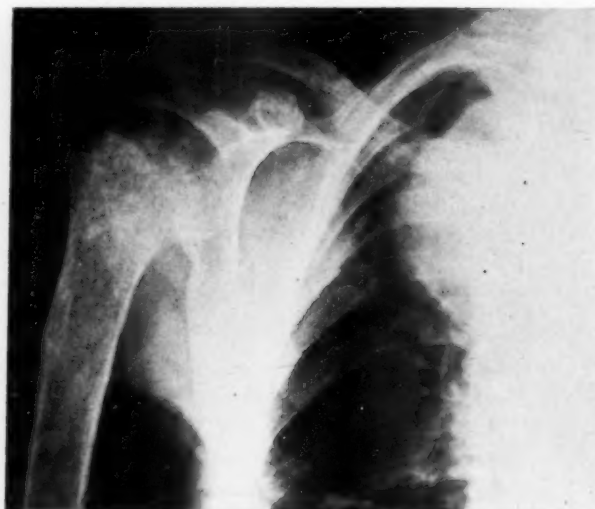


FIG. 6. Film of right shoulder and humerus taken on February 25, 1957, showing extensive bone damage. There is a fracture of the fifth rib posteriorly and calcification of the brachial artery.

anastomosing cords of large round cells with a finely granular cytoplasm and pale, vesicular nuclei identical with the chief cells seen in section of the parathyroid tumors. (Figs. 8 and 9.)

In the abdomen a spherical cystic mass, similar to that in the chest although smaller in size, was noted at the tail of the pancreas. It also consisted of a firm, fibrous capsule surrounding a mass of old clotted blood. On section, the pancreas revealed multiple, pale, poorly circumscribed nodules in most of the lobules. Many lobules which appeared normal had a finely nodular consistency. On microscopic examination these consisted of aggregates of islet cell tissue arranged in branching cords. Occasional islets had a fibrous capsule and the appearance of true adenomas. No such cells were noted in the wall of the cystic mass, which consisted wholly of fibrous tissue with scattered calcium deposits.

Adjacent to the prostate but not obstructing the urethra there was a circumscribed mass surrounded by a fibrous capsule. Cut sections revealed whorled masses of muscle fibers and on microscopic examination this was considered to be a leiomyoma.

All the bones were soft and easily fractured; microscopic examination revealed a mosaic pattern of distorted trabeculae lining spaces filled with a very cellular fibrous tissue. While the great blood vessels appeared normal, the medium-sized arteries which were sectioned showed calcium encrustations on the elastic fibers of the media. In the kidney, in addition to severe pyelonephritis with multiple cortical abscesses, the cortex and medulla contained deposits of calcium and there was evidence of obstruction as indicated by tubular dilatation. No renal calculi were noted. There was no evidence of either old or recent peptic ulceration. Calcification was not present in either the stomach wall or the lungs.

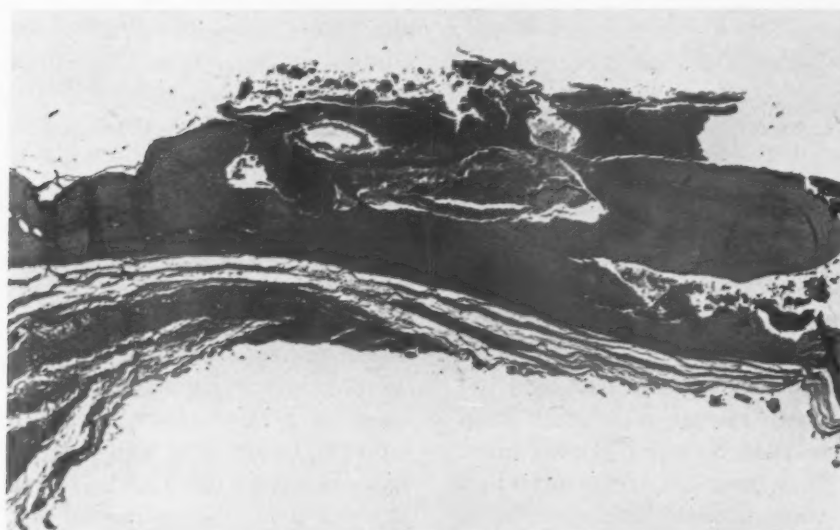


FIG. 8. Low power view of the mediastinal cyst wall, showing on its inner surface dark-staining aggregates of small uniform cells identical with those found in the parathyroid adenomas of the neck.

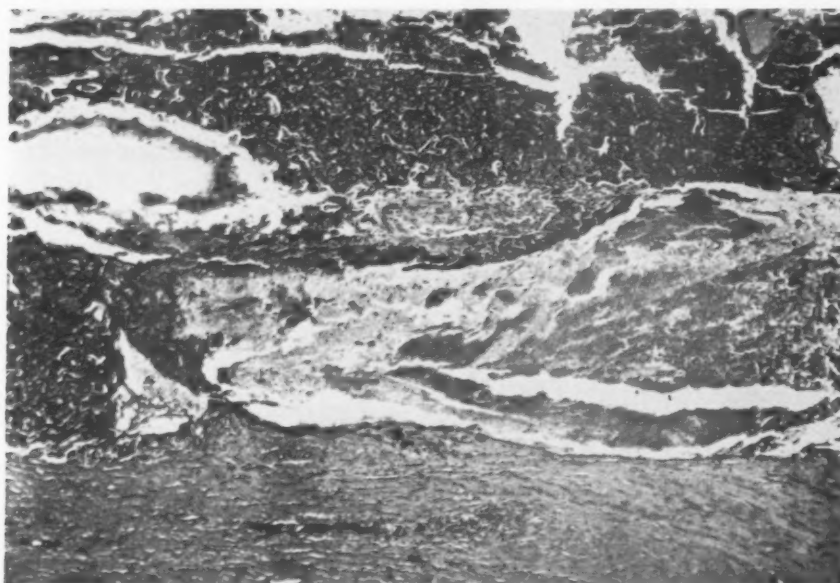


FIG. 9. Higher power of a portion of the mediastinal cyst, showing the debris of old hemorrhage adjacent to the remnants of parathyroid adenoma.

#### COMMENTS

The case herein described is the forty-seventh reported instance of multiple endocrine adenomas. It is notable in at least three respects: the size of the parathyroid tumor; the known duration of disease without symptomatic manifestation; and the diagnostic complications introduced before death by the presence of intercurrent renal failure.

Thirty-five of the forty-six reported cases of multiple endocrine adenomas involved the parathyroids. Twenty-three presented clinical

evidence of functionally active parathyroid tumors. Less than one-third of this latter group showed signs of generalized osteitis fibrosa cystica or metastatic calcification. All the tumors were relatively small, ranging in size from microscopic proportions to the grossly palpable. None were as massive as that in the present case.

Even in the numerous case reports of parathyroid tumors occurring alone, the majority of lesions tend to be relatively small. One report over a half century ago [17] described a tumor somewhat larger than the one herein recorded, involving the neck rather than the mediastinum.



The weight of the mass in the present case was never determined because of disintegration on sectioning, but the largest parathyroid tumor in a series reviewed by Castleman [12] was comparable in its over-all dimensions to that herein described and that tumor weighed 53 gm. The majority of tumors reported in this series weighed less than 4 gm. and tended to be solid tumors of neoplastic cells. Foci of hemorrhagic or fibrotic degeneration such as in the present case are uncommon.

The tumors in both the parathyroid and the pancreas in this case differed somewhat from those usually seen in either of these glands when they occur alone. The glandular overgrowth had the pattern of adenomatous transformation rather than tumor, consisting as it did of a proliferation of glandular elements in a nodular pattern without true encapsulation. This nodular hyperplasia was also noted in both adrenal cortices. Similar findings are reported in the majority of cases reviewed, although none presented evidence of clinical hyperadrenalism. In addition, none of the cases exhibited anatomic evidence of a parathyroid lesion either on roentgenograms or by palpation prior to the development of signs of hyperparathyroidism.

With regard to the question of duration and its relation to the natural history of this disease, it is not known nor can it be judged from the presence of any one tumor how many of the adenomas described, either in the parathyroids or the pancreas, were present at the time the mass was first discovered. It might be inferred from the morphological findings at postmortem that the course of the disease was favorably altered by the fortuitous occurrence of hemorrhage within the mass, at least partially disrupting the tumor or its circulation. The presence, however, of a low serum phosphorus when the patient was first examined suggests that either the mediastinal tumor was active at that time or that all the tumors were present and functional. If we accept the latter interpretation it is difficult to explain the relatively rapid development of symptomatic hyperparathyroidism during the last two years of his course. With the finding of one normal parathyroid gland, the possibility of supervening secondary hyperparathyroidism is unlikely. It appears, therefore, that the tumors were not only multicentric but dispersed in time as well.

The diagnosis of hyperparathyroidism in the presence of renal failure may be difficult. Serum

calcium and phosphorus levels are characteristically changed in one direction by hyperparathyroidism and in the other direction by renal disease. Normal values may result in this setting. In the present case, repeated serum calcium determinations were normal. The serum phosphorus, initially low, rose as renal failure became evident. Urinary calcium excretion studies during controlled calcium intake may be of help, but a low value in the presence of renal insufficiency, as obtained in this case, does not exclude hyperparathyroidism. Studies of calcium excretion may reflect depression of glomerular filtration rate rather than the functional state of the parathyroids [13]. The presence of a normal serum calcium in the face of chronic renal disease (with or without signs of ureteral stone, demineralization or metastatic calcification) should direct attention to the parathyroids [14].

#### SUMMARY

A case of multiple adenomas involving the parathyroids and pancreatic islets is presented. The earliest sign of disease was a large mediastinal mass later identified as a parathyroid adenoma.

The occurrence in this patient of a parathyroid tumor antedating by eight years the symptoms of hyperparathyroidism may add to our understanding of the natural history of this disease.

The literature is reviewed with special reference to hyperparathyroidism in this setting.

*Acknowledgment:* We are indebted to Henry G. Schmidt, M.D., of the Pathology Department, Bellevue Hospital, for reviewing the pathological material and to Paul Wermer, M.D., for his interest in this case.

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# The Primary Hypoventilation Syndrome\*

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**A**N interesting new syndrome of alveolar hypoventilation without underlying disease of the lungs or chest wall has recently emerged from the study of polycythemic patients in the pulmonary function laboratory.

Alveolar hypoventilation had previously been recognized as a complication of some other disease state, such as poliomyelitis in which there is a striking clinical picture that results from paralysis of the diaphragm and accessory muscles of respiration. It occurs as a late complication of other diseases of the neuromuscular apparatus such as amyotrophic lateral sclerosis and myasthenia gravis. Deformity and fixation of the chest wall, as in severe kyphoscoliosis or rheumatoid spondylitis, occasionally causes mechanical restriction of respiration and alveolar hypoventilation. Among the chronic lung diseases, alveolar hypoventilation is found in pulmonary emphysema. In this disease large areas of ventilated but poorly perfused lung tissue increase the physiologic dead space, so that a greater tidal volume must be moved to provide normal alveolar ventilation. In addition, this must be accomplished at a greater mechanical and metabolic cost because of the airway obstruction and the need to use inefficient accessory muscles of respiration. As the disease progresses the energy requirement of breathing becomes so great that ventilation is reduced. Two other clinical examples of hypoventilation are deep general anesthesia and depression of the respiratory center by such agents as narcotics and barbiturates.

All these clinical situations and the secondary hypoventilation which complicates them are readily recognized. When there is doubt, simple ventilatory studies demonstrate marked impairment.

A more obscure form of secondary hypoventilation is the cardiopulmonary syndrome of obesity. In 1951 Newman, Feltman and Devlin [7] reported detailed studies on five patients

with the clinical diagnosis of primary polycythemia. Arterial oxygen unsaturation was noted in two of these patients. No underlying cardiovascular or pulmonary abnormality was found and no satisfactory explanation was given for the unsaturation. It was incidentally noted that these two patients were excessively obese. Four years later the relationship of obesity, arterial oxygen unsaturation, and polycythemia was again noted and a cardiopulmonary syndrome associated with extreme obesity was postulated. This syndrome is being reported with increasing frequency [2-8]. Its clinical and laboratory features have been documented, and it has received recognition as an unusual, but not rare, entity. It is characterized by extreme obesity, cyanosis, polycythemia, right-sided heart failure, and central nervous system symptomatology such as headache, easy fatigability, somnolence, and muscular twitching. Normally, the mechanical work of breathing is made up of the elastic resistance of the lungs and chest and the resistance to flow in the airways. The frictional resistance of the tissues is negligible. It is theorized that, in extreme obesity, the deposition of fat in the chest and abdominal wall and in the abdominal viscera imposes a mechanical burden on the bellows action of the chest and diaphragm by increasing the usually small frictional resistance of the tissues. To minimize the work expended, ventilation is reduced, the constancy of the internal environment is sacrificed, and arterial oxygen unsaturation and carbon dioxide retention result. The syndrome is entirely reversible by weight reduction.

More recently another variant of the hypoventilation syndrome has evolved. It is similar in all respects to the cardiopulmonary syndrome of obesity except that the patients are not obese. In the absence of lung disease, obesity, and disease of the musculoskeletal apparatus of the chest, the syndrome has been attributed to

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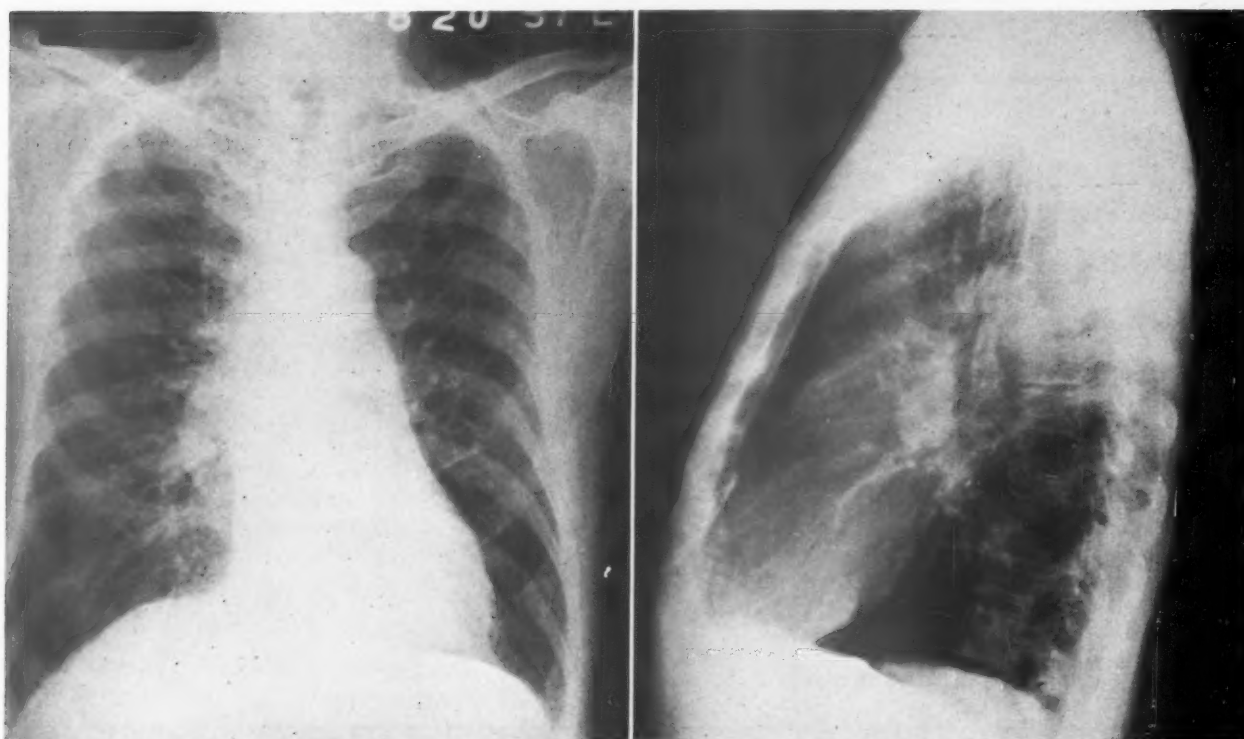


FIG. 1. Posteroanterior and lateral roentgenograms of the chest showing slight cardiac enlargement and passive pulmonary congestion. No evidence of lung disease.

disease of the medullary respiratory center. Four such cases have been reported [9-12].

We have recently studied a patient who appears to be an example of this primary hypoventilation syndrome. Special studies were made to attempt to clarify the nature of the defect in respiratory regulation.

#### CASE REPORT

A sixty-two year old white man entered the hospital with a two-year history of progressive easy fatigability, vertigo, and intermittent edema of the ankles. He complained of difficulty in staying awake. There were no respiratory symptoms. Two years prior to this hospitalization the patient was told that he had a positive Wassermann test and was treated with penicillin.

On admission, physical examination revealed somnolence, slight pitting edema of the ankles, and cyanosis of the nail beds and mucous membranes which was more intense when the patient was sleeping. Neurological examination revealed no abnormalities. The hemoglobin was 19.1 gm./100 cc.; hematocrit, 61 per cent; leukocyte count, 6,600/cu. mm., with a normal differential. The urinalysis was negative. Blood urea nitrogen, serum uric acid, postprandial blood sugar, serum sodium, potassium, and chloride were normal. The serum  $\text{CO}_2$  (venous blood) was 34 mEq./L. Results of liver function tests were normal. The Kolmer test for syphilis was posi-

tive in both the serum and spinal fluid. There was an elevation in the mid-zone of the colloidal gold curve. Examination of spinal fluid revealed no cells and spinal fluid protein was 34 mg. per cent. An electrocardiogram showed a vertical electrical axis with clockwise rotation and prominent P waves in leads II, III and aVf. X-ray examination of the chest (Fig. 1) showed moderate cardiac enlargement with no specific chamber enlargement. The pulmonary vasculature was prominent but there was no roentgenographic evidence of primary lung disease. X-ray films of the skull were normal. An electroencephalogram showed slight non-specific generalized abnormalities in both the waking and sleeping record.

The patient was treated with venesection of 1,500 cc. of blood, intermittent positive pressure breathing, digitalis and diuretics. On this regimen there was remarkable subjective improvement.

The pulmonary function studies which were carried out to establish the diagnosis are tabulated in Tables I to III. The lung volume studies were made with a 13.5 L. Collins respirometer. The index of intrapulmonary mixing and functional residual capacity were performed in duplicate by the open circuit oxygen inhalation method [13], for which the expired air was collected in a 125 L. Tissot gasometer and analyzed with a Waters nitrogen gas analyzer. Flow rates were measured by a pneumotachygraph standardized by rotameters in connection with a strain gauge transducer and electronic recorder. Expired

TABLE I  
LUNG VOLUMES

Pulmonary Function Test	Pre- dicted Normal	July 12, 1957		August 19, 1957		November 8, 1957	
		Meas- ured Value	% of Pre- dicted Normal	Meas- ured Value	% of Pre- dicted Normal	Meas- ured Value	% of Pre- dicted Normal
Inspiratory capacity (L.)	2.47	2.03	82	2.04	83	2.19	89
Expiratory reserve volume	1.01	.70	69	.80	79	.88	87
One second vital capacity (%)	>75	55	..	45	..	65	..
Three second vital capacity (%)	>90	84	..	77	..	88	..
Total vital capacity (L.)	3.48	2.73	78	2.84	82	3.07	88
7 minute nitrogen washout (%)	<2.5	..	..	2	..	3	..
Residual volume (L.)	2.43	..	..	2.3	94	2.23	92
Total lung capacity (L.)	5.9	..	..	5.14	87	5.30	90
Residual volume/total lung capacity	.41	..	..	.44	..	.42	..
Maximum breathing capacity (L./min.)	72	58.7	85	59	85	63.2	88
Air velocity index	1.0	1.1	..	1.0	..	1.0	..
Maximum inspiratory flow rate (L./min.)	>200	..	..	..	..	189	95
Maximum expiratory flow rate (L./min.)	>300	..	..	..	..	240	80

gases were analyzed for O<sub>2</sub> and CO<sub>2</sub> by the micro-Scholander technic [14]. Arterial oxygen content, oxygen capacity, and CO<sub>2</sub> content were determined using the Van Slyke and Neill [15] manometric apparatus. Arterial pH was measured at 37°C. with a Cambridge electron-ray research model pH meter. CO<sub>2</sub> tensions were calculated from the line charts of Van Slyke and Sendroy [16] and from the Henderson-Hasselbach equation. Arterial oxygen tensions were measured directly by the Riley bubble technic [17] and duplicates were required to check within 5 mm. Hg. Alveolar oxygen tensions were calculated on the basis of the alveolar gas equation. Minute ventilation was measured by collecting the expired air in a Tissot respirometer. The respiratory rate was recorded on a moving kymogram. Alveolar ventilation and physiologic dead space were calculated on the basis of the Bohr equation.

## RESULTS

The lung volumes (Table I) are low normal. Although there is some slight airway obstruction, manifested by a three second vital capacity less than 90 per cent, the possibility of pulmonary emphysema severe enough to produce respiratory acidosis is ruled out by the maximum breathing capacity of 85 per cent and the normal nitrogen washout and residual volume.

Oxygen tension and diffusion studies are outlined in Table II. The alveolar to arterial gradient is slightly elevated but is decreased with a lower oxygen concentration suggesting that the

gradient is due to venous admixture rather than a membrane defect. The diffusing capacity for oxygen is normal.

The detailed studies of the transfer of respiratory gases (Table III) establish the presence of alveolar hypoventilation. With only slight voluntary hyperventilation (increase in minute ventilation from 5.68 to 12.07 L./minute compared to a maximum breathing capacity of 59 L./minute) the patient was able to increase his arterial oxygen saturation from 72.8 per cent to 91.5 per cent, decrease his arterial CO<sub>2</sub> content from 67.4 volumes per cent to 61.2 volumes per cent, decrease his arterial pCO<sub>2</sub> from 70 mm. Hg to 58 mm. Hg and raise his pH from 7.33 to 7.38. This respiratory effort produced no dyspnea or discomfort.

TABLE II  
OXYGEN TENSION AND DIFFUSION STUDIES

% O <sub>2</sub>	pO <sub>2</sub> Inspired Air (mm. Hg)	pO <sub>2</sub> Alveolar Air (mm. Hg)	Arterial pO <sub>2</sub> (mm. Hg)	Gradient Inspired Air to Alveolar Air (mm. Hg)	Gradient Alveolar Air to Arterial Blood (mm. Hg)
18.55	132	45	30	87	15
22.64	162	83	61	79	22

DLO<sub>2</sub> = 17.9 ml./mm. Hg/min.

Normal DLO<sub>2</sub> = >15 ml./mm. Hg/min.

DLO<sub>2</sub> = diffusing capacity of the lung for oxygen.

TABLE III  
TRANSFER OF RESPIRATORY GASES

Respiratory Volumes and Arterial Blood Gases	Normal Resting Values	Resting	Voluntary Hyperven- tilation	Exercise	Sleep- ing	Breathing 100% O <sub>2</sub>
Respiratory rate (per minute).....	12-16	17	17	.....	.....	.....
Minute ventilation (L./min.).....	6	5.68	12.07	14.2	.....	5.82
Alveolar ventilation (L./min.).....	4.2	2.35	7.55	3.08	.....	.....
Alveolar ventilation (L./min./M <sup>2</sup> ).....	2.3	1.3	4.2	1.7	.....	.....
CO <sub>2</sub> output (cc./min./M <sup>2</sup> ).....	110	102	.....	140	.....	.....
O <sub>2</sub> intake (cc./min./M <sup>2</sup> ).....	140	138	.....	212	.....	.....
Gas exchange ratio.....	.85	.74	.....	.66	.....	.....
O <sub>2</sub> capacity (vol. %) arterial blood.....	21	24.81	.....	25.70	.....	.....
O <sub>2</sub> content (vol. %) arterial blood.....	20	18.07	22.70	15.28	14.41	25.51
O <sub>2</sub> saturation (%) arterial blood.....	95	72.8	91.5	59.5	58.1	100 + .70
CO <sub>2</sub> content (vol. %) arterial blood.....	50	67.4	61.2	67.1	67.3	64.1
CO <sub>2</sub> tension (mm. Hg) (calculated) arterial blood..	40	70	58	75	72	71
pH.....	7.40	7.33	7.38	7.29	7.31	7.30

The normal response to exercise is a reflex increase in ventilation of such magnitude that there is no drop in the arterial oxygen saturation and only slight changes in the pH and arterial pCO<sub>2</sub>. In our patient although the minute ventilation increased from 5.68 to 14.2 L./minute the alveolar ventilation increased only from 2.35 to 3.08 L./minute. This indicates that the increase was in rate rather than in depth with a disproportionate increase in dead space ventilation. This inadequate response was accompanied by a drop in arterial oxygen saturation from 72.8 per cent to 59.5 per cent with an increase in arterial pCO<sub>2</sub> from 70 mm. Hg to 75 mm. Hg and a drop in pH from 7.33 to 7.29.

During a lull in the test the patient fell asleep. Cheyne-Stokes respirations were noted. An arterial blood specimen withdrawn without arousing the patient had an arterial oxygen saturation of 58.1 per cent with a correspondingly elevated CO<sub>2</sub> and lowered pH.

In chronic respiratory acidosis the usual response to the administration of high concentrations of oxygen is depression of respiration [18-22]. The respiratory center has become tolerant to an elevated arterial pCO<sub>2</sub> and the primary chemical stimulus to respiration is hypoxia. The removal of this stimulus by the administration of oxygen is followed by a decrease in minute and alveolar ventilation, further elevation of the arterial pCO<sub>2</sub> and depression of the pH. Occasionally carbon dioxide narcosis, coma, and death ensue.

Such a response was recorded in a patient with the primary hypoventilation syndrome by Pare and Lowenstein [10] who noted a decrease in alveolar ventilation from 1.24 L./minute per square meter body surface area to 0.4 L. following the administration of 100 per cent oxygen. This was accompanied by an increase in arterial pCO<sub>2</sub> from 60 to 72 and a decrease in arterial pH from 7.4 to 7.33. The patient reported from the Massachusetts General Hospital [11] went into coma during the administration of oxygen by nasal catheter. CO<sub>2</sub> retention was demonstrated and the sensorium improved following withdrawal of oxygen. Ratto et al. [9] and Richter et al. [12] do not mention the ventilatory response to oxygen in their patients. In our preliminary studies a paradoxical response to the administration of 100 per cent oxygen was noted. Instead of depression there was a slight increase in minute ventilation from 5.68 to 5.82 L. To confirm this surprising finding and further clarify the defect in the regulatory mechanism of our patient, studies of the response to respiratory stimuli were carried out. (Table IV.) These are presented graphically in Figures 2 and 3. The patient's response to varying concentrations of oxygen ranging from 14 per cent to 100 per cent was studied on four different days. On three of the four days he was also given one or more concentrations of CO<sub>2</sub> in oxygen. On one occasion he was given a large dose of intravenous lobeline, a chemoreceptor stimulant, and 500 mg. of aminophylline intravenously. Each time the paradoxical response to the



TABLE IV  
RESPONSE TO RESPIRATORY STIMULI

Respiratory Volumes and Arterial Blood Gases	July 31, 1957			August 7, 1957					August 19, 1957						October 7, 1957									
	18 % O <sub>2</sub>	20 % O <sub>3</sub>	100 % O <sub>2</sub>	15 % O <sub>2</sub>	18 % O <sub>2</sub>	20 % O <sub>2</sub>	5 % CO <sub>2</sub> 95 % O <sub>2</sub>	13 16	13 13	13 13	13 13	13 13	12 5.81	Lobe- line 10 mg. I.V.	100 % O <sub>2</sub>	5 % CO <sub>2</sub> 95 % O <sub>2</sub>	15 % O <sub>2</sub> Amino- phylline 500 mg. I.V.	14 % O <sub>2</sub>	18 % O <sub>3</sub>	23 % O <sub>2</sub>	30 % O <sub>2</sub>	4 % CO <sub>2</sub> 96 % O <sub>2</sub>	6 % CO <sub>2</sub> 94 % O <sub>2</sub>	8.5 % CO <sub>2</sub> 91.5 % O <sub>2</sub>
Respiratory rate.....	14	16	15	11	12	12	16	13	13	13	13	13	12	12	15	15	12	13	15	17	17	14	15	18
Minute ventilation (L./min.).....	6.73	7.24	7.95	5.8	7.1	8.1	16.5	5.55	5.66	5.67	5.95	6.46	5.81	6.44	14.84	8.69	8.69	7.18	7.85	8.62	8.95	12.0	15	18.5
Alveolar ventilation (L./min.).....	3.99	4.10	5.01	3.64	4.75	5.75	13.36	3.00	3.11	3.12	3.40	3.91	3.46	3.50	11.90	6.54	6.54	4.63	4.91	5.29	5.62	9.26	12.06	14.97
O <sub>2</sub> capacity (vol. %)	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04
O <sub>2</sub> content (vol. %)	16.86	16.59	21.59	17.8	17.8	17.8	20.1	9.86	13.95	18.36	18.36	18.36	18.36	18.36	20.57	20.81	16.44	9.58	12.11	16.57	17.58	19.94	20.28	19.95
O <sub>2</sub> saturation (%)	80.1	78.9	>100	85.7	85.7	85.7	97.1	50.7	71.8	94.5	94.5	94.5	94.5	94.5	>100	>100	84.6	49.3	62.3	85.3	90.5	>100	>100	>100
CO <sub>2</sub> content (vol. %)	63.4	63.4	62.6	63.7	63.7	63.7	62.8	66.9	65.3	64.3	64.3	64.3	64.3	64.3	62.6	65.5	55.9	65.7	67.4	66.3	65.7	67.1	66.4	70.0
CO <sub>2</sub> tension (mm. Hg) (calculated)	58	59	59	61	61	61	63	61	61	62	62	62	62	62	60	66	38	60	67	66	65	67	68	75
pH.....	7.37	7.36	7.36	7.35	7.35	7.35	7.35	7.35	7.36	7.34	7.34	7.34	7.34	7.34	7.35	7.32	7.51	7.36	7.32	7.32	7.33	7.34	7.31	7.29

administration of oxygen was noted; with increasing concentrations of oxygen in the inspired air, minute and alveolar ventilation increased. In Figure 2 the values obtained in this patient for oxygen saturation are plotted on the abscissa and minute ventilation on the ordinate. Similar data obtained from two other patients with hypoxemia at rest, one with pulmonary emphysema and one with pulmonary sarcoidosis, are plotted for comparison. It can be seen that in the patients with emphysema and sarcoidosis the removal of the hypoxic drive to respiration is followed by a diminution in minute volume. This is the anticipated response to the removal of a potent chemical stimulus. In contrast, the withdrawal of the hypoxic stimulus was followed in our patient by an increase in minute volume. To the best of our knowledge this paradoxical response to the removal of the hypoxic drive is unique in clinical medicine. It resembles the response seen in experimental animals in which the nerves from the chemoreceptors in the carotid body and aortic arch are severed [23-37]. Hypoxia stimulates respiration reflexly through the chemoreceptors. It is a depressant when it acts directly on the medullary center in the absence of the reflex drive. In animals with denervated chemoreceptors, the removal of this depressant effect by relief of hypoxia increases the sensitivity of the respiratory center to CO<sub>2</sub>. The studies outlined herein seem to indicate that our patient does not respond to hypoxia as a respiratory stimulant. On two occasions arterial oxygen saturations of 49.3 per cent and 50.7 per cent resulted in a minute ventilation less than that at higher oxygen saturations. The highest minute ventilation was consistently recorded on inhalation of 100 per cent oxygen. The administration of 10 mg. of lobeline intravenously failed to increase the minute ventilation. This also suggests the presence of an inadequate chemoreceptor response.

In chronic respiratory acidosis the response to increased concentrations of CO<sub>2</sub> in the inspired air is diminished [32-37]. The decreased sensitivity of the respiratory center is established by demonstrating the presence of an elevated arterial CO<sub>2</sub>. Quantitative evaluation of the ventilatory response to CO<sub>2</sub> must be related to changes in arterial pCO<sub>2</sub> rather than to concentration of CO<sub>2</sub> administered. The normal subject will double his alveolar ventilation in response to an increase of 1.5 mm. Hg in the

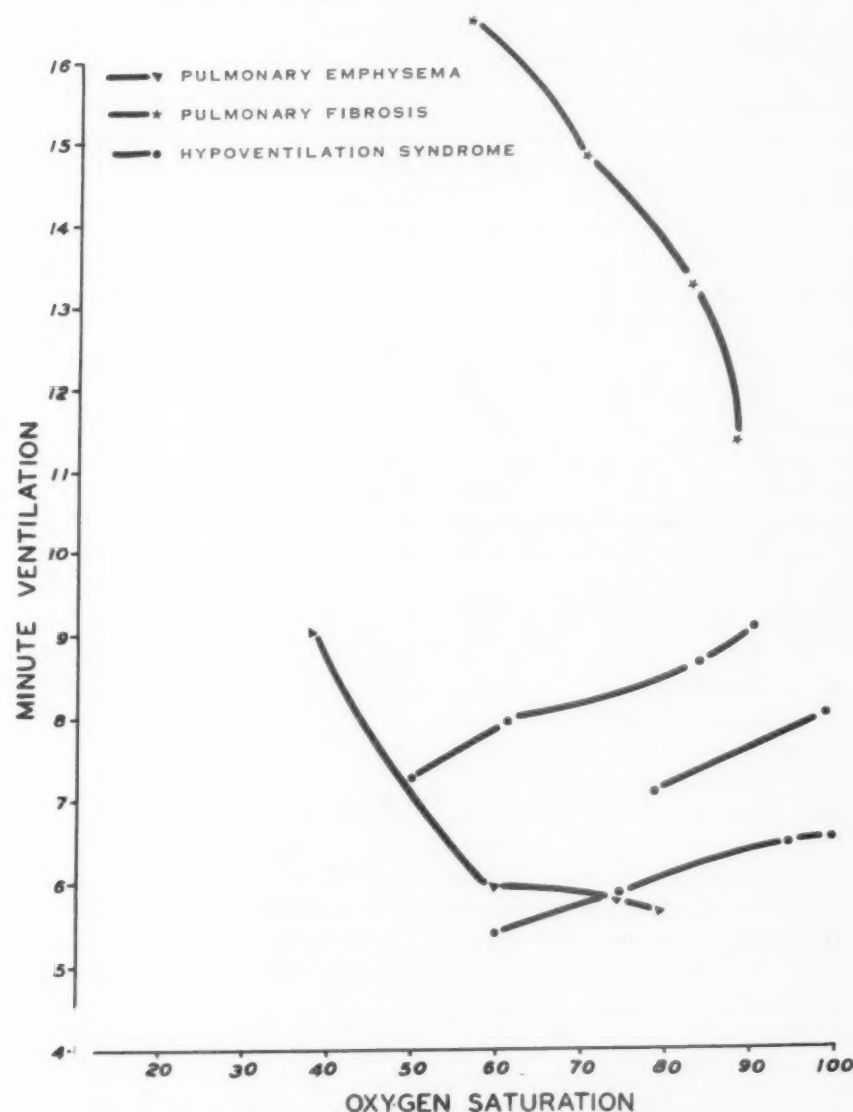


FIG. 2. The hypoxic drive to respiration. Oxygen saturation on the abscissa, minute ventilation on the ordinate.

arterial  $p\text{CO}_2$  [37]. Ratto [9] reported that the administration of 7.5 per cent  $\text{CO}_2$  to a patient with the primary hypoventilation syndrome produced an increase in minute ventilation to 14 to 21 L. This appears to be a diminished response but since no arterial blood studies were reported a definite interpretation is not justified. Pare and Lowenstein [10] reported an increase in alveolar ventilation from 1.24 to 2.3 L./minute per square meter body surface area in response to the inhalation of 5 per cent  $\text{CO}_2$  in 30 per cent  $\text{O}_2$ . There was a change in arterial  $p\text{CO}_2$  from 58 to 69 mm. Hg indicating a diminished response to  $\text{CO}_2$ . Richter et al. [12] reported no change in minute ventilation on inhalation of 3 per cent and 5 per cent  $\text{CO}_2$  although the arterial  $p\text{CO}_2$  rose from 64 to 80

mm. Hg and the pH dropped from 7.34 to 7.27. The administration of 7.5 per cent  $\text{CO}_2$  to the patient in the Massachusetts General Hospital produced a minute ventilation of 20 L./minute [11]. No arterial blood studies were reported. Our patient's response to increased concentrations of  $\text{CO}_2$  in the inspired air is charted graphically in Figure 3. Arterial  $\text{CO}_2$  tension is plotted on the abscissa and minute ventilation on the ordinate. The responses of a normal subject and of a patient with severe chronic pulmonary emphysema with alveolar hypoventilation and  $\text{CO}_2$  retention are also shown. The normal respiratory center is exquisitely sensitive to slight increases in the partial pressure of  $\text{CO}_2$  in its environment. As shown in Figure 3 the slope of the normal response is almost a straight vertical

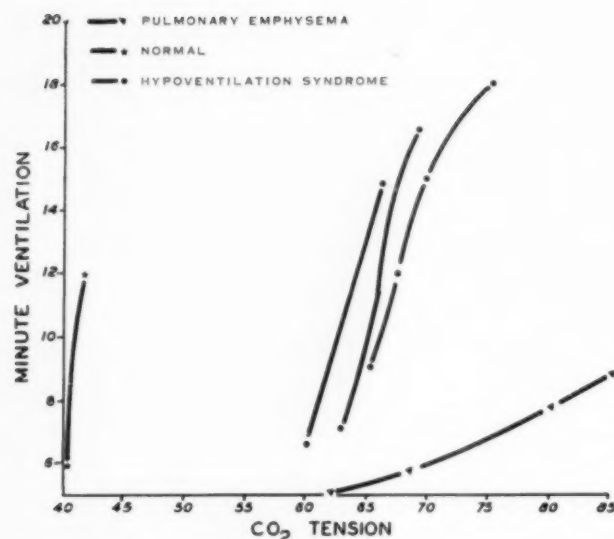


FIG. 3. The hypercapnic drive to respiration. CO<sub>2</sub> tension on the abscissa, minute ventilation on the ordinate.

line. An increase of arterial pCO<sub>2</sub> as little as 1.5 mm. Hg will normally double the minute volume of ventilation. In contrast, the patient with chronic respiratory acidosis, whose respiratory center is tolerant to increased levels of CO<sub>2</sub> pressure, responds to a further increase of 22 mm. Hg in his arterial pCO<sub>2</sub> with only slight increase in minute ventilation. Our patient, whose initial arterial blood studies were indistinguishable from those of the patient with respiratory acidosis, showed only a slightly diminished response to CO<sub>2</sub>.

The responses to respiratory stimuli in the five patients with this syndrome are summarized in Table v. Responses in our patient were characterized by a diminished but appreciable response to CO<sub>2</sub>, a totally absent response to hypoxia and lobeline, and a very vigorous response to aminophylline which is a potent stimulant to the medullary center. This would seem to indicate a chemoreceptor defect with no

TABLE v\*  
RESPONSE TO RESPIRATORY STIMULI

Authors	Exercise	Increased CO <sub>2</sub>	Change in O <sub>2</sub> Concentration	Adrenalin®	Breath Holding	I.V. Lobeline	Central Nervous System Stimulant
Ratto et al. [9] . . .	(Data not given) arterial O <sub>2</sub> saturation ↓	M.V. ↑ to 14 to 21 L. (no arterial blood studies) on 7.5% CO <sub>2</sub>	.....	No change in ventilation	Held for 90 seconds without discomfort with arterial O <sub>2</sub> saturation to 35% arterial pCO <sub>2</sub> ↑ to 77	.....	.....
Pare and Lowenstein [10]	.....	A.V. ↑ from 1.24 L./min./M <sup>2</sup> to 2.3 with arterial pCO <sub>2</sub> increase from 60 to 72 mm. Hg on 5% CO <sub>2</sub>	A.V. ↓ from 1.24 L./min./M <sup>2</sup> to .4 L./min./M <sup>2</sup> on 100% O <sub>2</sub>	.....	.....	.....	.....
Richter et al. [12]	Arterial O <sub>2</sub> saturation ↓, arterial pCO <sub>2</sub> ↑, pH ↓	No change in M.V. on 3% and 5% CO <sub>2</sub> in spite of change in arterial pCO <sub>2</sub> from 64 to 80	.....	.....	.....	.....	.....
M. G. H. [11] No. 865180	Arterial O <sub>2</sub> saturation ↑ from 77% to 86%; no dyspnea	M.V. ↑ to 20 L./min. on 7.5% CO <sub>2</sub> ; no arterial blood studies	Stupor progressing to coma on administration of O <sub>2</sub> ; improvement in sensorium on withdrawal	.....	.....	.....	No change in M.V. following caffeine or I.V. sodium salicylate
Rodman and Close	Arterial O <sub>2</sub> saturation ↓, arterial pCO <sub>2</sub> ↑, pH ↓	Increase in ventilation intermediate between normal and pulmonary emphysema on 4%, 6%, 8% CO <sub>2</sub>	↑ In ventilation with increasing concentrations of oxygen	.....	.....	No change in ventilation	↑ In ventilation with drop in pCO <sub>2</sub> from 63 to 38 mm. Hg. and ↑ in pH from 7.35 to 7.51 after 500 mg. aminophylline I.V.

\* M.V. = minute ventilation.  
A.V. = alveolar ventilation.  
I.V. = intravenous.



impairment of the center itself. Since chemoreceptors are present in both carotid regions and in the aortic arch, however, it seems unlikely that a disease process could be so widespread yet selective. In addition, animal experiments have demonstrated that section of the afferent nerves from the chemoreceptors is not followed by the marked derangement seen in our patient. It seems more reasonable to think that the process is central in location, either in the pathways of the chemoreceptor impulses to the center or in the center itself. Some physiologists speak of a chemical respiratory center which is sensitive to changes in  $p\text{CO}_2$  and pH and a reflex respiratory center which mediates impulses from the chemoreceptors, cerebrum, muscles and joints, and from the stretch organs that originate the Hering-Breuer reflex [38]. Although there is no anatomical evidence for such a dichotomy, if two such distinct regions exist, a lesion of the reflex respiratory center would satisfactorily explain the findings in our patient.

#### COMMENTS

The most prominent clinical feature of patients with the primary hypoventilation syndrome is polycythemia. The differentiation between primary and secondary polycythemia is often relatively clear from the clinical picture. There may be an obvious cause of arterial oxygen unsaturation, or the presence of leukocytosis, thrombocytosis and splenomegaly may suggest that the hematologic problem is a primary one. It seems likely, however, that unless oxygen saturation studies are carried out, cases of the primary hypoventilation syndrome, in which the presenting picture is that of polycythemia, will be misdiagnosed as primary polycythemia.

When the diagnosis of secondary polycythemia has been established by the presence of arterial oxygen unsaturation, other causes for the arterial unsaturation must be excluded. The factors which must be considered include: (1) right-to-left shunts; (2) diffusion defects; and (3) alveolar hypoventilation, (A) secondary to disease of the lungs, chest wall or neuromuscular apparatus and (B) primary.

An intracardiac right-to-left shunt is almost always associated with other findings which point to its presence. Occasionally a pulmonary arteriovenous fistula will be associated with a minimum of other findings. The presence of a shunt can usually be established by failure to

achieve 100 per cent oxygen saturation of arterial blood after administration of 100 per cent oxygen for thirty minutes. Unsaturation due to either a diffusion defect or alveolar hypoventilation will be corrected by this procedure.

Certain types of lung disease, such as pulmonary sarcoidosis and fibrosis, are characterized pathologically by diffuse interstitial fibrosis with thickening of the alveolar-capillary membrane resulting in impaired diffusion of oxygen from the alveolus into the pulmonary capillary. Arterial unsaturation and polycythemia are common. The roentgenographic appearance of the lungs in such patients is usually quite suggestive. In addition, differential diagnosis is facilitated by the observation that there is no concomitant elevation of the arterial  $\text{CO}_2$  content or depression of the pH.  $\text{CO}_2$  is twenty times more diffusible than oxygen and death normally occurs from hypoxia long before there could be  $\text{CO}_2$  retention.

The biochemical hallmarks of alveolar hypoventilation are arterial oxygen unsaturation and hypercapnia with a low or normal pH. The presence of  $\text{CO}_2$  retention in association with the oxygen unsaturation rules out both a right-to-left shunt and a diffusion defect.

When all patients with alveolar hypoventilation have been categorized into recognized disease entities, there remains a small group, of which our patient is the fifth to be reported, in whom the disease has been placed by deduction in the medullary respiratory center. To the best of our knowledge, this localization has not yet received anatomical confirmation. Ratto and his associates [9] postulated vascular thromboses in the brain stem of their patient, although there were no associated neurologic signs. Pare and Lowenstein [10] attributed the findings in their patient to "abnormal function of the respiratory center," but did not suggest an etiologic diagnosis. Richter, West and Fishman [12] proposed the possibility of encephalitic damage on the basis of a suggestive history. The patient reported in the medical grand rounds of the Massachusetts General Hospital [17] had no evidence of primary disease of the central nervous system. Our patient had a history of syphilis and a positive spinal fluid serology but there were no other signs of disease of the central nervous system. Etiologic diagnosis must await postmortem study.

With increasing awareness of this syndrome and with increasing availability of pulmonary

function laboratories it appears likely that primary hypoventilation will be recognized more frequently. The study of these patients with disturbed regulation of respiration may well shed light on the normal regulatory mechanism.

## SUMMARY

Until recently alveolar hypoventilation had been recognized only as a complication of such disease states as poliomyelitis, amyotrophic lateral sclerosis, severe kyphoscoliosis, pulmonary emphysema, or depression of the central nervous system. In 1955 a more obscure form of secondary hypoventilation was recognized as an unusual complication of extreme obesity. In the past three years four patients with a "primary hypoventilation syndrome" have been reported in whom there was no underlying disease of the lungs or musculoskeletal apparatus of the chest. Disease of the respiratory center has been postulated to explain the findings.

In this paper a fifth patient with the "primary hypoventilation syndrome" is reported. The findings in the other four patients and in alveolar hypoventilation in general are reviewed.

Special studies were carried out to clarify the defect in the respiratory regulatory mechanism. These studies demonstrated a totally absent response to hypoxia and lobeline and a slightly diminished response to CO<sub>2</sub>. The administration of oxygen was followed by a paradoxical increase in minute ventilation which was explained on the basis of increase in sensitivity of the respiratory center to CO<sub>2</sub> with improved oxygenation. The findings are compared to those in experimental animals in which the afferent nerves from the chemoreceptors are severed.

The problems of the differential diagnosis of polycythemia is discussed and the place of the pulmonary function laboratory in solving the problem is emphasized.

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# Hyperthyroidism Associated with Renal Tubular Acidosis\*

## *Discussion of Possible Relationship*

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THE patient described in this report presented evidence of two syndromes, hyperthyroidism and renal tubular acidosis (hyperchloremic acidosis due to tubular insufficiency without glomerular insufficiency), apparently not before reported in this association. The possibility that the renal tubular acidosis represented a sequel to the patient's initial episode of hyperthyroidism seems to us to be the most likely explanation of this association. This paper will report the evolution of the patient's clinical problem with the evidence available to support this hypothesis.

### CASE REPORT

The patient (M. B.), a forty-four year old white woman, was hospitalized for the first time from September 23 to October 10, 1954. At the time of admission to the Wilkes-Barre General Hospital the patient complained of agitation, depression, vomiting, weakness, thirst, frequency of urination and palpitation of the heart. She had been well until the spring of 1954 when her father died suddenly of a coronary occlusion. Shortly thereafter the patient noted palpitation of the heart and tremor of her hands. These symptoms continued and were aggravated by the two months of illness and death of her mother from pyelonephritis and terminal uremia in September 1954. She then became extremely anxious and agitation with depression developed. She slept continuously for twelve hours at a time and began to vomit on arising each morning despite a good appetite. At the same time there was weakness, thirst and frequency of urination. The patient also noticed some weakness of the quadriceps muscles on climbing stairs. There was no history of alkali or vitamin D ingestion. Her menstrual periods became scanty and were associated with irritability, depression and occasional hot flushes. In August 1954 her weight was 110 pounds; by Septem-

ber 1954 it had fallen to 88 pounds. Her pulse rate during this period remained elevated at about 112/minute. System review was otherwise within normal limits. The past medical history disclosed an appendectomy in 1928; anal fissurectomy in 1943; tonsillectomy during childhood; migraine for years; and psoriasis at ages twenty to thirty. No pregnancies had occurred.

The patient's mother died at the age of seventy of pyelonephritis with uremia. She had had thyrotoxicosis (treated by thyroidectomy), coronary artery disease, gallstones and migraine. Her father died at the age of seventy-two of a myocardial infarction. One living sister, aged forty, has migraine and gallstones. Another sister, aged forty-six, has hypertension. A brother died when 3 days old of trauma occurring at birth. Another sister died at the age of one year of infantile diarrhea but prior to death had shown normal development. Her husband is living and well. The social history was non-contributory.

The patient's vital signs were: temperature, 98°F.; pulse, 112; blood pressure, 108/56 mm. Hg; respirations, 20. She was prematurely gray and extremely wasted in appearance. Her eyes were prominent with infrequent blinking and staring, but no other eye signs were noted.

At this admission the results of the following studies were found to be within normal limits or negative: Hemoglobin, red cell count, white cell count, cholecystogram, upper gastrointestinal series, and intravenous urogram. A forty-eight-hour eosinophil response test using 40 units of ACTH administered intramuscularly daily produced a fall in circulating eosinophils from 132/cu. mm. to 66/cu. mm. and a rise of urinary 17 ketosteroids from 10.3 mg./twenty-four hours to 34.1 mg./twenty-four hours. The Kepler-Power water excretion test was positive with a night volume of 500 ml., a maximal day volume of 170 ml. and an A value of 7.6. Her eosinophils were 5 per cent of a white blood cell count of 8,800/cu. mm. Urinalysis showed nothing abnormal except for a

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TABLE I  
SERUM ANALYSES AND THYROID FUNCTION TEST VALUES IN PATIENT M. B.

Date	Serum						Blood Urea Nitrogen (mg./100 ml.)	Fasting Blood Sugar (mg./100 ml.)	Protein (gm./100 ml.)	Cholesterol (mg./100 ml.)	Alkaline Phosphatase (Shinowara Units)	Basal Metabolic Rate (%)	Protein-bound Iodine (mg. %)	Comments
	Na (mEq./L.)	K (mEq./L.)	Cl (mEq./L.)	CO <sub>2</sub> (mM./L.)	Ca (mg./100 ml.)	P (mg./100 ml.)								
	(134-144)	(3.5-5.3)	(97-108)	(24-31)	(9.0-11.5)	(2.5-4.4)	(8-18)	(65-105)	(6.3-8.0) (Alb. 3.4-5.0) (Glob. 2.1-4.1)	(90-290)	(2.2-8.5)		(4.0-6.1)	
10/8/54	136	3.6	...	...	...	...	25	72	...	...	8.0	+22	...	Thyrototoxicosis, complete heart block; potassium, alkali and anti-thyroid therapy
11/1/54	140	1.2	117	13.8	13.4	3.9	29	106	5.5	155	...	...	...	
11/3/54	146	3.1	113	12.6	11.4	...	...	...	...	...	...	...	...	
11/4/54	148	1.5	117	14.8	...	...	24	...	...	...	...	...	...	
11/5/54	142	2.4	115	14.4	10.5	...	...	...	...	...	...	...	...	
11/6/54	...	3.1	...	16.0	...	...	...	...	...	...	...	...	...	
11/10/54	144	5.0	109	26.1	11.0	3.6	12	...	...	...	...	+26	...	
11/13/54	142	4.8	105	28.4	...	4.4	...	...	...	...	...	...	...	
11/16/54	...	...	...	...	10.5	...	...	...	...	...	...	...	...	
11/17/54	144	4.4	105	28.0	10.3	4.0	...	...	...	...	...	...	...	
3/1/55	...	...	...	...	10.0	4.0	20	...	...	...	...	+1	...	
3/9/55	...	4.0	...	...	10.0	...	20	...	...	250	...	...	...	
3/14/55	141	3.6	112	15.1	9.5	2.9	15	98	3.9/2.5	...	6.7	...	...	
3/17/55	138	...	114	12.1	...	...	...	...	...	243	4.5	...	...	Thyroidectomy
3/23/55	138	3.8	114	14.9	...	...	...	...	...	...	...	...	...	Postoperative alkali therapy
3/24/55	130	2.6	102	15.0	...	...	9	...	...	...	...	...	...	
3/25/55	135	2.9	104	23.1	8.5	1.1	6	...	...	...	...	...	...	Alkali therapy started
3/26/55	...	3.2	...	33.4	...	...	...	...	...	...	...	...	...	
3/28/55	143	4.3	101	29.2	8.9	2.0	...	...	...	...	...	...	...	4/15/55 (after NH <sub>4</sub> Cl test 4/1/55)
5/17/55	...	...	...	24.4	9.4	2.8	...	...	...	...	...	...	...	
1/29/56	144	...	120	15.0	...	2.9	16	92	...	...	4.7	...	...	Second NH <sub>4</sub> Cl test; alkali therapy stopped three weeks before
1/31/56	...	3.7	...	...	...	...	...	...	4.1/2.4	214	...	...	...	
2/1/56	138	3.4	119	13.8	9.8	3.1	...	...	...	...	4.6	...	...	
2/4/56	...	...	...	...	10.2	3.1	...	...	...	214	...	...	...	
2/6/56	...	...	...	...	9.2	2.1	...	...	...	...	...	-3	6.3	
4/6/57	142	4.3	103	20.0	9.8	3.5	9	83	...	...	5.1	...	8.2	Third NH <sub>4</sub> Cl test; alkali therapy stopped one week before admission
4/11/57	139	4.9	111	21.1	...	...	...	...	...	...	...	...	...	

NOTE: Figures in parentheses represent normal values.



FIG. 1. Portion of a roentgenogram showing nephrocalcinosis. Small, less dense areas of calcification not reproducible in this copy were also present.

trace of albumin on one occasion and a fixed specific gravity of 1.001 to 1.002. Gastric analysis showed a histamine-fast achlorhydria. The chest roentgenogram revealed a small heart. The figures obtained for the various serum electrolytes and renal function studies are shown in Table 1. At this time the differential diagnosis was presumed to be that of either salt-losing nephritis, Addison's disease or a toxic goiter. During hospitalization she had received three doses of 25 mg. of cortisone at four-hour intervals; following this she looked brighter, began to eat better and felt stronger. She was then referred to the Hospital of the University of Pennsylvania for further studies.

The patient was hospitalized for the second time from October 29 to November 24, 1954. During the interval between the two hospitalizations intermittent swelling of the ankles had developed and her physician noted enlargement of her thyroid gland which previously had not been palpable. On system review at the time of admission to the Hospital of the University of Pennsylvania the only new abnormality noted was an absent menstrual period immediately prior to admission.

In addition to the previous findings the physical examination now showed enlargement of the thyroid, which was approximately three to four times normal

size. The following studies were performed and found to be either normal or negative; hemoglobin, white cell count, chest roentgenogram and serological tests for syphilis. Her significant laboratory studies have been summarized in Table 1. Urinalysis showed 1-plus albumin and 1-plus sugar. Abdominal or urographic roentgen examinations were not performed during this admission.

During the day following admission the patient continued to be quite nauseated and vomited frequently; therefore therapy with intravenous fluids was begun. On November 1, a complete heart block developed with ventricular premature contractions. At this time treatment was begun with cortisone, administered intramuscularly; a low calcium diet; and intravenous administration of sodium iodide. Because of the probable association of the heart block with hypokalemia, potassium chloride was given intravenously. On November 2 the heart block ceased, but despite the use of potassium chloride intravenously her serum potassium was 1.5 mEq./L. On November 5, following continued therapy with intravenous potassium chloride, physiological saline solution, sodium iodide and oral fluids, her condition improved both in terms of her general appearance and of the electrolyte studies. (Table 1.) At this time the presumptive diagnosis was hyperthyroidism with secondary hypercalcemia; and it was assumed that polydipsia, polyuria, nausea and vomiting had developed secondary to the hypercalcemia, with resultant potassium depletion. The patient was given Lugol's solution, 20 drops three times a day, from November 6 to November 15. On November 11 a regimen of methimazole, 15 mg. administered orally every eight hours, was instituted. Her first weight on November 8 following this clinical crisis was 81 pounds. She was discharged from the hospital on November 24, following a recuperative period. Methimazole, 10 mg. orally every eight hours, was continued along with sodium amytal and a therapeutic vitamin formula. Her thyroid gland had increased in size during the time of hospitalization and it was decided to remove it surgically at a later date.

Following discharge from the hospital, the patient did well; the dosage of methimazole was reduced to 2.5 mg. per day without a return of symptoms. Administration of Lugol's solution was started again on February 5, 1955, preparatory for surgery.

The patient's third hospitalization was from March 13 to March 30, 1955. The patient was readmitted to the Hospital of the University of Pennsylvania for a subtotal thyroidectomy. She felt well and had noted a return of menses.

The patient's vital signs were: blood pressure, 110/78 mm. Hg; pulse, 84; respirations, 19; temperature, 98°F. There was no change in her physical condition except for the development of urticarial lesions which were believed to be the result of a reaction to penicillin given for a respiratory infection a



TABLE II  
AMMONIUM CHLORIDE TEST OF RENAL ACIDIFICATION FUNCTION (PATIENT M. B.)

Blood Acid-Base—Cutaneous ("Arterialized") Whole Blood																		
Urine Values (per 24 hours)																		
	NH <sub>4</sub> Cl Dose (mEq./day)	Vol- ume (ml.)	Specific Gravity	pH	Na (mEq.)	K (mEq.)	Ca (mEq.)	NH <sub>4</sub> <sup>+</sup> (mEq.)	Titra- ble Acidity (mEq.)	CO <sub>2</sub> (mM)	Cl (mEq.)	pH	CO <sub>2</sub> (mM./L.)	pCO <sub>2</sub> (mm.Hg)	Buffer Base (mEq.)	Hema- to- crit	Hemo- globin (mM./L.)	
First Test 1955																		
Control	3/30-31	1260	1.011	7.6	125	57	5	6	...	22	91	(7.35-7.45)	(22 ± 2)	(40 ± 5)	(49 ± 3)	...	...	
	3/31-4/1	1580	1.010	7.51	149	79	6	7	...	25	146	7.43	16.8	29.5	43	0.420	8.1	
	Average	...	...	...	137	68	6	7	...	...	119	...	...	...	...	...	...	
Acid stress	4/1-2	166*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
	4/2-3	166*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
	4/3-4	111	1.011	6.45	130	133	13	37	20	25	226	...	...	...	...	...	...	
Change from control	...	...	...	...	-7	+65	+7	+30	+20	...	+107	...	...	...	...	...	...	
Changes in cations as % of Cl change	...	...	...	...	$\frac{\Delta \text{NH}_4 + \Delta \text{TA}}{\Delta \text{Cl}} = 47\%$					$\frac{\Delta \text{Na} + \Delta \text{K} + \Delta \text{Ca}}{\Delta \text{Cl}} = 61\%$					...			
Second Test 1956																		
Control	1/30-31	1700	1.009	6.70	88	77	12	24	13	21	101	...	...	...	...	...	...	
	1/31-2/1	1695	1.008	6.70	72	63	10	22	11	10	69	7.33	11.6	27.0	37	0.454	8.5	
	Average	...	...	...	80	70	11	23	12	...	85	...	...	...	...	...	...	
Acid stress	2/1-2	166*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
	2/2-3	166*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
	2/3-4	111	1.009	6.42	84	109	24	38	19	7	173	7.18	10.0	30.0	30	0.445	9.0	
Change from control	...	...	...	...	+4	+39	...	+15	+7	...	+88	...	...	...	...	...	...	
Changes in cations as % of Cl change	...	...	...	...	$\frac{\Delta \text{NH}_4 + \Delta \text{TA}}{\Delta \text{Cl}} = 25\%$					$\frac{\Delta \text{Na} + \Delta \text{K} + \Delta \text{Ca}}{\Delta \text{Cl}} = 64\%$					...			
Third Test 1957																		
Control	4/8-9	1510	1.012	6.72	91	68	...	25	11	8	69	7.30	14.8	34.5	40	0.47	9.6	
	4/9-10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
Acid stress	4/10-11	187*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
	4/11-12	2510	1.010	6.40	130	132	...	49	32	7	203	7.29	15.1	37.0	40	0.50	9.7	
Change from control	...	...	...	...	+39	+64	...	+24	+21	-1	+134	...	...	...	...	...	...	
Changes in cations as % of Cl change	...	...	...	...	$\frac{\Delta \text{NH}_4 + \Delta \text{TA}}{\Delta \text{Cl}} = 34\%$					$\frac{\Delta \text{Na} + \Delta \text{K} + \Delta \text{Ca}}{\Delta \text{Cl}} = 77\%$					...			

Note: Figures in parentheses represent normal values. In tests on patient M. B., her sister M. D. and a control subject, the ammonium chloride dose of 6.0 gm./M<sup>2</sup> body surface area per day of Talbot et al. [6] was used. Patient M. B. was unable to take the full dose on the third day of her first test; therefore, the second test was conducted in the same way. In her third test, no ammonium chloride was given on the third test day. In the test of sister C. W., the dose of Albright and Reifenstein [6] was used. Detailed standards for the interpretation of the ammonium chloride test are not available. We have taken an increase of ammonium (NH<sub>4</sub><sup>+</sup>) and titratable acidity (T. A.) of 60 per cent or more of the increase in chloride excretion (see Control Subject) and a urinary pH below 6.0 as minimum standard of adequate renal performance. The control subject ingested a much larger dose of NH<sub>4</sub>Cl than did patient M. B. On Talbot's dosage, the kidney may not be able to prevent the development of systemic metabolic acidosis.

\* 6.0 gm. NH<sub>4</sub>Cl/m<sup>2</sup> body surface area.

week prior to admission. The weight had increased to 116 pounds at this time, and she felt generally well. The laboratory findings are summarized on Table I. The following additional studies were made and found to be either normal or negative: hemoglobin, electrocardiogram, chest roentgenogram. A urogram showed evidence of renal calcification present in the renal pyramids of both kidneys. (Fig. 1.) This finding had not been evident on the urograms taken in October 1954 prior to the discovery of the hypercalcemia.

Thyroidectomy, with removal of 80 per cent of the gland tissue, was performed on March 23, 1955, by Dr. William Fitts. At that time, the thyroid gland was found to be nodular and enlarged. The pathological report was as follows: "The sections reveal numerous, variable-sized follicles lined by cuboidal epithelium, some flat and others high cuboidal. Within the lumina is colloid. There is a prominence of the connective tissue stroma dividing the gland into nodular-like areas. In some areas there is evidence of lymphocytic invasion. Diagnosis: Toxic nodular goiter."

Except for a mild fall in the serum potassium level following operation (Table I), the patient did well. In view of the persistent acidosis that the patient had experienced, a renal tubular lesion producing chronic metabolic acidosis with postassium wasting was now suspected. To confirm this diagnosis an ammonium chloride test of renal acidification function was performed. The results of this test are summarized in Table II. This demonstrated that the patient was unable to meet the demands of the acid load by a sufficient increase in titratable acid and ammonia, with resultant cation wastage, largely of potassium. Therefore an alkalinizing salt mixture designed to supply approximately 50 mEq. of cation per day, 40 mEq. as potassium and 10 mEq. of sodium, was prescribed.

The patient's fourth hospitalization was from January 29 to February 7, 1956. The patient continued to have polyuria and polydipsia since her last admission even though she faithfully took the alkaline mixture. For the purposes of evaluation she stopped her medication three weeks before entering the Hospital of the University of Pennsylvania. Since the previous admission, she had noted a return of migraine headaches, a decrease in duration of her menstrual periods and hot flushes. No abnormal physical findings were noted during this admission.

Laboratory studies were as follows: hemoglobin, 15.2 gm. per cent, white blood cell count, 7,400/cu. mm., with a normal differential count. Routine urinalysis was normal. An electrocardiogram was reported within normal limits except for slightly smaller T waves than on March 30, 1955. A chest roentgenogram was normal. An intravenous urogram revealed multiple punctate calcifications of both kidneys as previously described. No progression of the process was noted. Table I summarizes the additional laboratory data of that admission.

TABLE III  
RENAL FUNCTION VALUES ON PATIENT M. B.

Date	Specific Gravity (18 hr. conc. test)	Urine pH	Creatinine Clearance (ml./min./ 1.73m <sup>2</sup> S.A.)	PSP Excretion (%)	
				15 min.	2 hr.
11/5/54.....	.....	5.82	64	..	..
11/10/54.....	.....	.....	..	20	..
11/17/54.....	.....	6.52	95	..	..
11/19/54.....	1.010 (12 hr.)	.....	..	..	..
3/14/55.....	1.010	.....	62	27	..
9/23/55.....	1.018	.....	..	..	..
11/14/55.....	1.012	.....	..	..	..
1/31/56.....	.....	6.7	75	..	..
2/4/56.....	.....	.....	87	27	76
4/8/57.....	1.016 (24 hr.)	.....	86	30	80

The ammonium chloride test of renal acidification function was repeated and the results are summarized in Table II. There had been no improvement in the basic defect, namely an inability to acidify the urine and adequately to increase ammonia excretion (with resultant cation wastage). The defect in concentrating ability continued. The menstrual changes with hot flushes were considered to be due to the menopause. The patient was discharged and instructed to continue taking her alkalinizing mixture.

The patient was hospitalized for the fifth time from April 5 to April 13, 1957. After the previous admission the patient had a recurrence of symptoms which were again suggestive of hyperthyroidism. These consisted of increasing nervousness, hand tremor, weight loss, excessive perspiration, prominence of the eyes, and a return of thyroid swelling. A new symptom, intermittent pain in various anterior and posterior locations in the chest, appeared. Psoriasis, present years ago, reappeared three months before this admission to the Hospital of the University of Pennsylvania. Physical findings of significance included a regular tachycardia, a papulosquamous lesion on the left elbow, eye signs of hyperthyroidism, diffuse enlargement of the thyroid gland, and slightly tender cystic areas in the breasts. The significant laboratory studies are included in Tables I, II and III. A roentgenogram of the abdomen again showed a nephrocalcinosis of about the same degree as noted previously.

All the evidence suggested a recurrence of hyperthyroidism, and therapy with I<sup>131</sup> was begun. The laboratory data (Tables I and III) showed that the syndrome of renal tubular acidosis was still present (alkali therapy had been stopped one week before admission) and therapy with alkalinizing potassium and sodium citrate was reinstituted.

#### METHODS

The laboratory studies were performed with methods used in the Chemical Section of the Department

of Medicine as previously reported [1,2] and in the Pepper Laboratory of the Hospital of the University of Pennsylvania.

The acid-base determinations were made on cutaneous whole blood "arterialized" by preheating of the forearm in hot water and were analyzed by the micromethod of Singer et al. [3]. This method determines  $\text{CO}_2$  content, pH, hematocrit and hemoglobin concentration by direct analysis, and  $\text{pCO}_2$  and buffer base by nomographic estimation [4].

The ammonium chloride tests were carried out with two dosage regimens. One was in amounts approximating the 130 mEq./day, used by Albright [5], and the other was the dose of 6 gm.  $\text{NH}_4\text{Cl}$ /square meter of body surface area/day as recommended by Talbot et al. [6]. The tests were run for three or five days as noted in Table II.

*Comment:* The patient was apparently in good health, except for occasional attacks of migraine, until the age of forty-four. At this time symptoms developed which, by November 1954, six months after onset, were clearly attributable to hyperthyroidism. Treatment at this point brought the thyrotoxic problem under control. After appropriate preparation, the patient withstood a subtotal thyroidectomy for removal of a toxic nodular goiter in March 1955.

At the time of the second admission the patient's blood chemistry values were compatible with the presence of renal tubular acidosis with accompanying potassium depletion (low serum  $\text{CO}_2$ , high serum chloride, low serum potassium), but this diagnosis was tentative because of the presence of other disturbances such as inadequate food intake with vomiting for several months, the possibility of moderate glomerular insufficiency (blood urea nitrogen 24 to 29 mg. per cent), and a urine pH of 5.8. Adequate hydration, and potassium and alkali therapy corrected all the chemical disturbances.

At the third admission for her thyroidectomy in March 1955, the chemical signs of renal tubular acidosis (low serum  $\text{CO}_2$  content and hyperchloremia) were again present. The blood urea nitrogen level was slightly elevated (20 mg./100 ml.) but this mild azotemia was completely inadequate to account for a metabolic acidosis. The diagnosis of renal tubular acidosis seemed certain, but further pertinent studies were postponed until after her thyroidectomy. The patient was treated with a sodium-potassium citrate mixture after thyroidectomy with a resulting normal serum  $\text{CO}_2$  level. The alkali therapy was discontinued three days before the first ammonium chloride test of renal acidification function.

(Table II.) The plan for the test included administration of 6.0 gm. of  $\text{NH}_4\text{Cl}$  per square meter of body surface area per day (166 mEq./day) but the patient was unable to take the full dose on the third day because of nausea. As shown in Table II, during this first test the patient's increase in chloride excretion above control levels was accompanied predominantly by fixed cation excretion (60 per cent) since the increase in cation-sparers, titratable acidity and ammonia minus bicarbonate, was only 51 per cent. This response would be considered inadequate even in the presence of normal blood acid-base values [6];\* the existence of a mild metabolic acidosis at the onset of the test, as well as an insufficient fall in urine pH, adds weight to the presumption of an inadequate renal response to the acid stress. Therefore, a defect in renal capacity for adequate acid excretion and/or bicarbonate reabsorption was established as the basis for the metabolic acidosis. At this time creatinine clearance (Table II) was slightly depressed and the concentrating power of the kidney was limited to a specific gravity of 1.010 (eighteen-hour test); however, these functional impairments were attributed to the effects of prior potassium depletion [8].

The patient was again treated with a sodium-potassium citrate prescription. Twice again within the next two years the alkalinizing mixture was discontinued temporarily for retesting with ammonium chloride and both times metabolic acidosis recurred and the patient failed to show adequate function of renal acidification. (Tables I and II.) Nevertheless, the concentrating power improved and the creatinine clearance was maintained at high levels. The nephrocalcinosis continued to be present, without evidence of change in its severity.

A remark by the patient that her two sisters, the only living blood relatives, had polyuria led us to investigate the possibility that they also had the syndrome of renal tubular acidosis. Venous serum analysis showed that they had normal concentrations of serum chloride, total  $\text{CO}_2$  content, and creatinine. Urinary concentration tests (eighteen hours) showed terminal specific gravities of 1.022 and 1.018. The sister showing the value of 1.018 was found to have eaten watermelon and cantaloupe during the conduct

\* A subsequent study of indices of acid excretion [7] has shown that this index is not definitive; nevertheless, by the more definitive criteria there established this patient clearly has a tubular defect in acid excretion.



TABLE IV  
AMMONIUM CHLORIDE TEST OF RENAL ACIDIFICATION  
PATIENT M. B.'S NORMAL SISTERS (M. D., G. W.) AND A NORMAL CONTROL SUBJECT

Urine Values (per 24 hours)										Blood Acid-Base—Cutaneous ("Arterialized") Whole Blood							
Date	NH <sub>4</sub> Cl Dose (mEq./day)	Vol- ume (ml.)	Specific Gravity	pH	Na (mEq.)	K (mEq.)	Ca (mEq.)	NH <sub>4</sub> <sup>+</sup> (mEq.)	Titrat- able Acidity (mEq.)	CO <sub>2</sub> (mM.)	Cl (mEq.)	pH	CO <sub>2</sub> (mM./L.)	pCO <sub>2</sub> (mm.Hg)	Buffer Base (mEq./L.)	Hema- to- crit	Hb (mM./L.)
Sister M. D.																	
Control.....	4/56	2330	1.005	5.90	132	61	10	33	29	2	126	(7.35-7.45)	(22±2)	(40±5)	(40±3)	0.435	8.7
Acid stress for 5 days.....	185*	2125	1.010	5.40	108	74	15	201	36	1	293	7.41	24.5	45	51.5	0.435	8.7
Change from control.....												7.30	13.4	32	39.5	0.503	8.8
Changes in cations as % of Cl change.....					-24	+13	+5	+168	+7		+167						
					$\frac{\Delta\text{NH}_4^+ + \Delta\text{TA}}{\Delta\text{Cl}} = 105\%$					$\frac{\Delta\text{Na} + \Delta\text{K} + \Delta\text{Ca}}{\Delta\text{Cl}} = 0\%$							
Sister C. W.																	
Control.....	7/56	1750	1.010	5.95	113	65	14	39	28		111	7.40	20.9	39	46.5	0.395	8.0
Acid stress for 3 days.....	112	1770	1.011	5.15	135	59	21	95	46		212	7.44	18.3	31.5	35.0	0.404	7.8
Change from control.....					+22	-6	+7	+56	+18		+101						
Changes in cations as % of Cl change.....					$\frac{\Delta\text{NH}_4^+ + \Delta\text{TA}}{\Delta\text{Cl}} = 75\%$					$\frac{\Delta\text{Na} + \Delta\text{K} + \Delta\text{Ca}}{\Delta\text{Cl}} = 23\%$							
Control Subject																	
Control.....		1005	1.023	5.97	162	72	10	36	29	3	163	7.40	19.4	37	46	0.441	
Acid stress 3rd day.....	217*	2385	1.012	5.00	204	125	19	156	59	3	413	7.31	13.5	32	40	0.495	
Change from control.....	217				+42	+53	+9	+123	+30		+250						
Change in cations as % of Cl change.....					$\frac{\Delta\text{NH}_4^+ + \Delta\text{TA}}{\Delta\text{Cl}} = 61\%$					$\frac{\Delta\text{Na} + \Delta\text{K} + \Delta\text{Ca}}{\Delta\text{Cl}} = 42\%$							

NOTE: Figures in parentheses represent normal values.  
\* 6.0 gm. NH<sub>4</sub>Cl/m<sup>2</sup> body surface area.

of the concentration test. Ammonium chloride tests in the two sisters (Table iv) showed that they had essentially normal renal responses to an acidification stimulus.

#### COMMENTS

The diagnoses of hyperthyroidism and renal tubular acidosis were clearly established in this patient, but whether or not any relation can be demonstrated between them is a matter for discussion.

Recent reviews of renal tubular acidosis have shown that patients with this syndrome can be divided into three main groups: infants and young children [9], adults with a familial incidence of the same disorder [10], and adults standing as apparently isolated cases [10,11]. The existence of the infantile group and the group with the familial trend have added strong weight to the belief that renal tubular acidosis is the result of an inherited defect in the renal capacity for acidification of the urine, but the possibility that some of the adult cases may represent development of the defect from a non-genetic cause in adult life has not been excluded.

Our patient enjoyed completely good health until the development of hyperthyroidism at the age of forty-four, without evidence of the disorders of bone metabolism, impaired growth, episodic paralysis or constitutional complaints which have heretofore characterized this disturbance [10-12]. Furthermore, nephrocalcinosis was not observed on intravenous urographic study early in October, 1954 but was found at the time of the third admission in March 1955. Therefore, it is possible that the nephrocalcinosis was a result of the rapid turnover and high urinary excretion of calcium which is known to be one of the metabolic manifestations of hyperthyroidism [13]. If the nephrocalcinosis developed during the first episode of hyperthyroidism it may, in this case, have been a cause of the syndrome of renal tubular acidosis rather than a sequel. Experimental study of renal calcification in the rat has shown that calcium may be precipitated either in the renal pelvis or in the lower portion of the renal tubules, depending on the amounts of calcium presented for excretion and on urine acidity [14]. The nephrocalcinosis in this patient's kidneys lies in the renal medulla and pyramids, known to be a major site of ammonia production [15]; a fact which fits well with the observation that this patient's failure in acid excretion is in part due to inadequate am-

monia production. It might be argued that failure of bicarbonate reabsorption and hydrogen excretion [16] proximal to the site of ammonia production could account for the failure of ammonia production. However, ample evidence exists to show that major increases in ammonia excretion can occur in the presence of an alkaline urine [17] if metabolic circumstances require it.

Certainly, renal tubular acidosis may be present unaccompanied by nephrocalcinosis [18] and the appearance of nephrocalcinosis cannot, by itself, be taken as proof that this patient did not have renal acidosis before its appearance. Furthermore, the incidence of symptomless renal tubular acidosis is unreported because for the most part only patients requiring hospital care have been described in the literature.

It is conceivable, although unproved, that the syndrome of renal tubular acidosis in this patient with hyperthyroidism resulted from nephrocalcinosis produced by hypercalcemia and hypercalciuria which developed as a complication of hyperthyroidism. The nephrocalcinosis, inducing tubular damage, led to impairment of the renal acidification mechanisms, and this impairment produced the syndrome of renal tubular acidosis. The evidence for this speculation lies largely in the facts that the patient lived a substantially healthy life to the age of forty-four, and that no historical or chemical evidence could be advanced for the presence of renal tubular acidosis in the patient's parents, two living sisters, and two dead siblings.

#### SUMMARY

In a forty-four year old woman, previously healthy, hyperthyroidism developed, following the death of her parents. She was found to have hypercalcemia, and a diagnosis of nephrocalcinosis with renal tubular acidosis was established after the hyperthyroidism was brought under control. Roentgenographic evidence of nephrocalcinosis developed during the hyperthyroid episode.

The response of the patient to an ammonium chloride test of renal acidification function was characteristic of patients with renal tubular acidosis; two sisters showed normal responses.

We offer as an explanation the suggestion that the renal tubular acidosis may have arisen as a result of tubular damage secondary to nephrocalcinosis which apparently developed during the most severe phase of the hyperthyroidism.

*Acknowledgment:* We acknowledge the kind cooperation of Dr. John G. Reinhold, Chemical Division, William Pepper Laboratory, Hospital of the University of Pennsylvania, in the performance of the clinical chemical determinations. The research chemistry was performed by Mrs. Lidia Kosolapovs, Mrs. Katharine Wisniewski, and Mr. James Mitchell of the Chemical Section of the Department of Medicine.

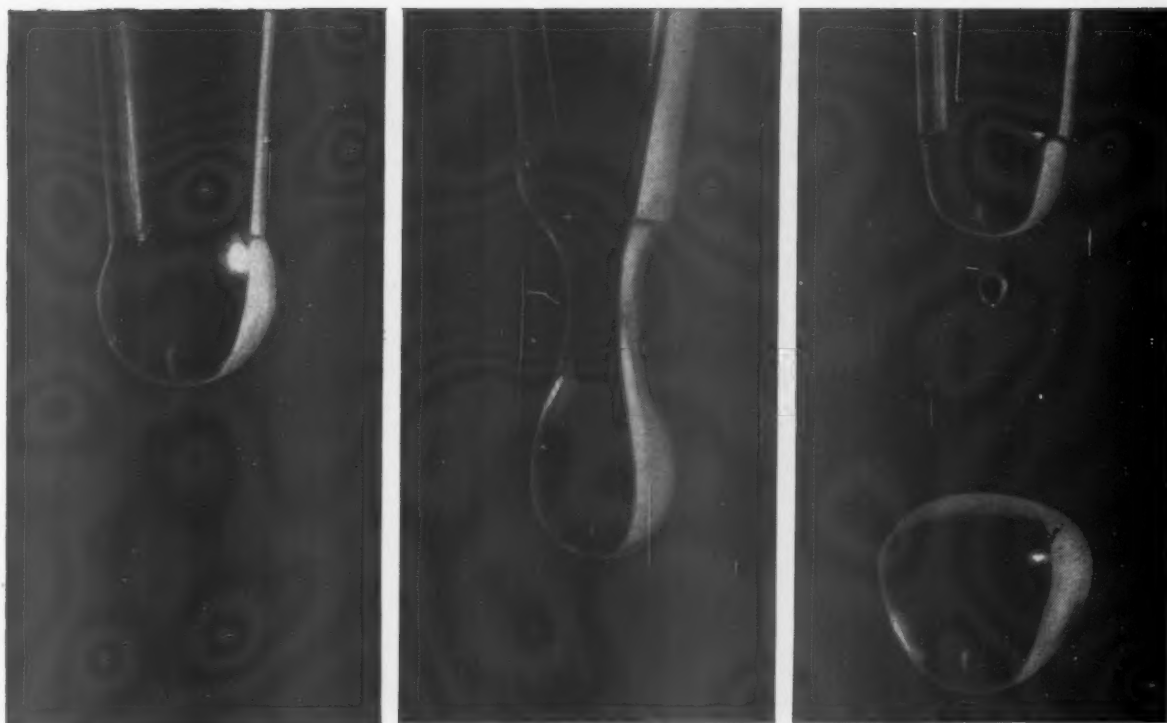
## ADDENDUM

During the patient's last visit (February 1959), one of us (E. J. H.) discovered that she has been taking only a third of the recommended alkaline prescription since 1955. This delinquency may explain the failure of the renal acidification function to improve and of the nephrocalcinosis to disappear.

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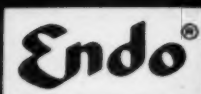
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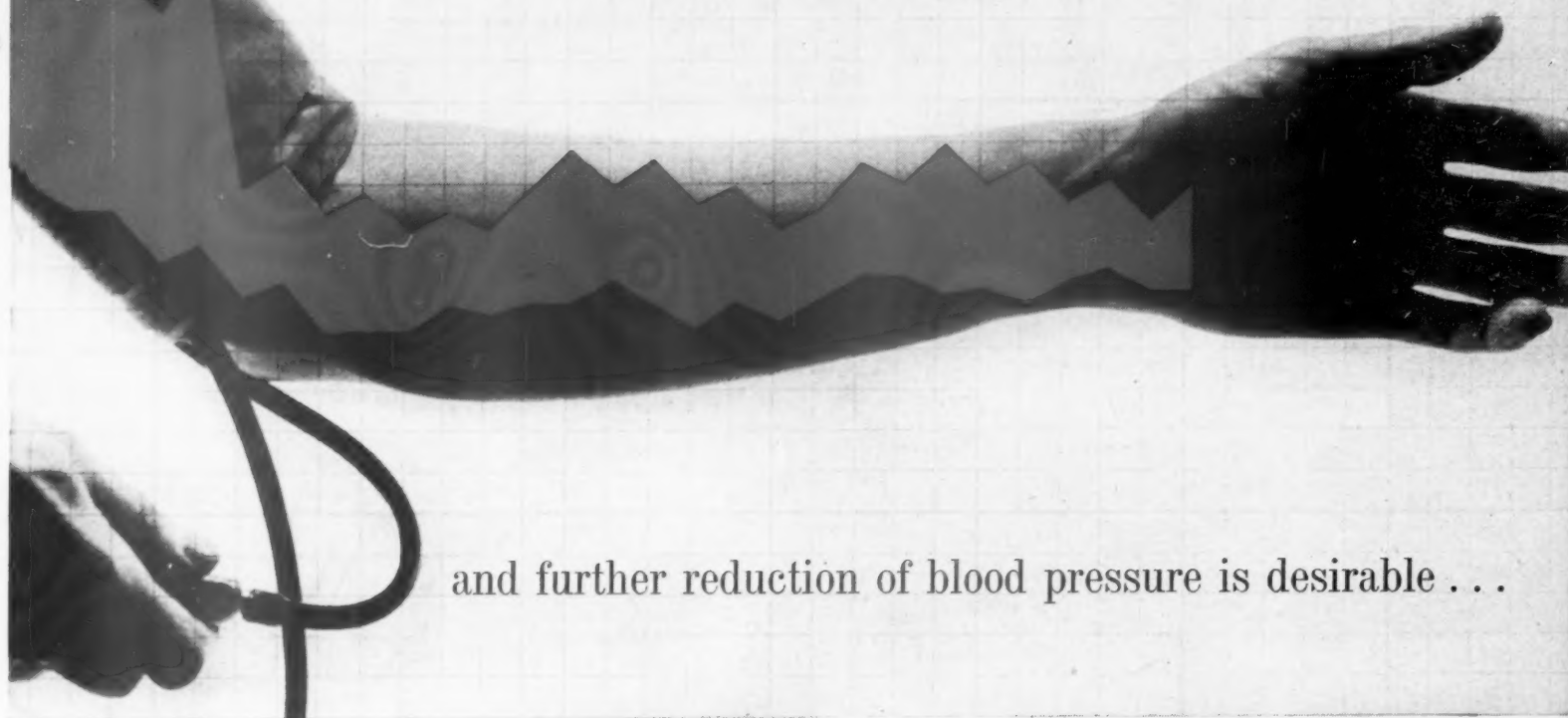
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**Dosage:** Usual starting dose is 1 tablet q.i.d. When necessary, this dose may be gradually increased up to 3 tablets q.i.d.

**Composition:** Each light-pink, scored tablet contains 1 mg. 2-diethylaminoethyl benzilate hydrochloride (benactyzine HCl) and 400 mg. meprobamate.


**References:**

1. Alexander, L.: J.A.M.A. 166:1019, March 1, 1958.
2. Current personal communications; in the files of Wallace Laboratories.
3. Pennington, V.M.: Am. J. Psychiat. 115:250, Sept. 1958.



**for depression**

**Deprol<sup>†</sup>**

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†TRADE-MARK UD-9182



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AROUND-THE-CLOCK  
CONTROL OF APPETITE**

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ENDURETS<sup>™</sup>  
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ENDURETS is a Geigy trademark.

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highest fluid yields,  
lowest blood pressure levels  
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**diuretic-antihypertensive**  
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(hydrochlorothiazide CIBA)

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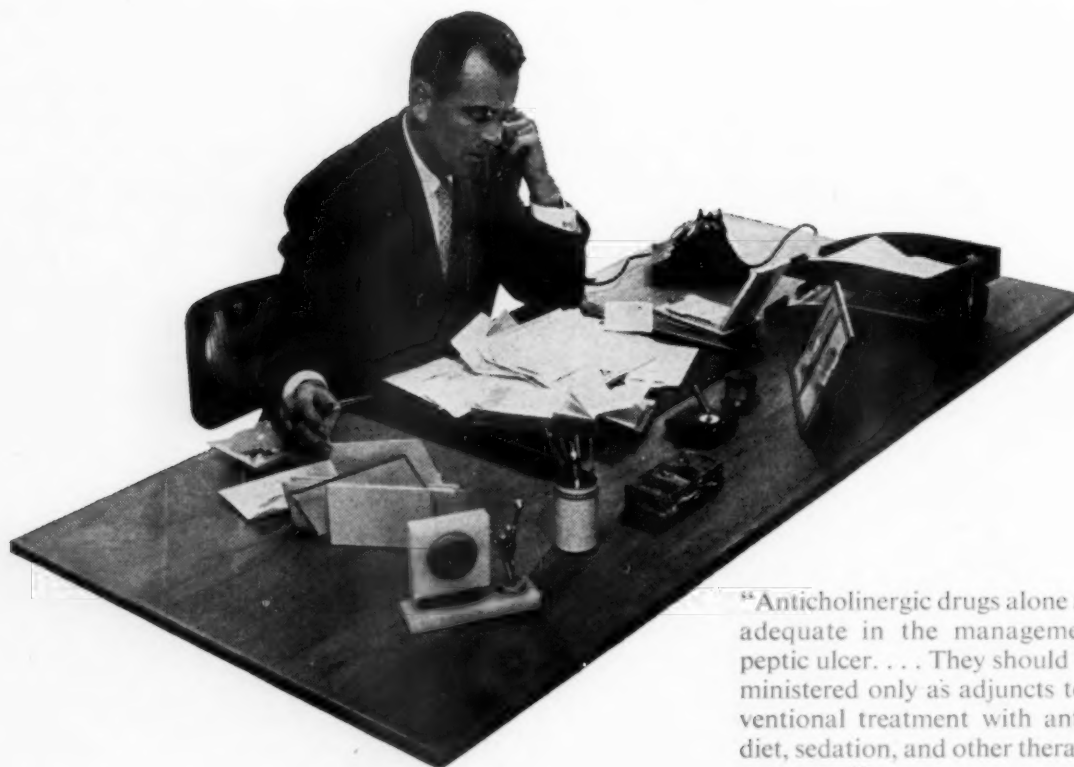
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"Anticholinergic drugs alone are inadequate in the management of peptic ulcer. . . . They should be administered only as adjuncts to conventional treatment with antacids, diet, sedation, and other therapeutic measures."<sup>1</sup>

1. Kirsner, J.B., et al.: M. Clin. North America 41:499 (March) 1957.

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**ALUDROX<sup>®</sup> SA<sup>\*</sup>**  
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<sup>\*</sup> Sedative and Anticholinergic

SUPPLIED: SUSPENSION, bottles of 12 fl. oz. TABLETS, bottles of 100. Each teaspoonful (5 cc.) and tablet contains 2.5 mg. of ambutonium and 8 mg. of butabarbital combined with aluminum hydroxide and magnesium hydroxide approximating 1 teaspoonful of aluminum hydroxide gel and 1/4 teaspoonful of milk of magnesia. Also available: Tablets Ambutonium Bromide, 10 mg., bottles of 100.

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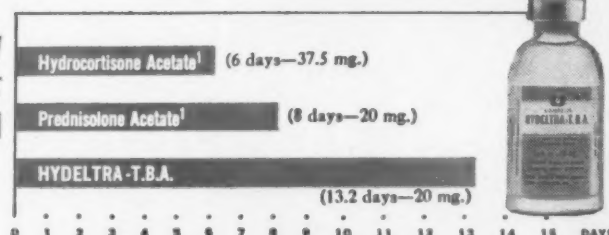
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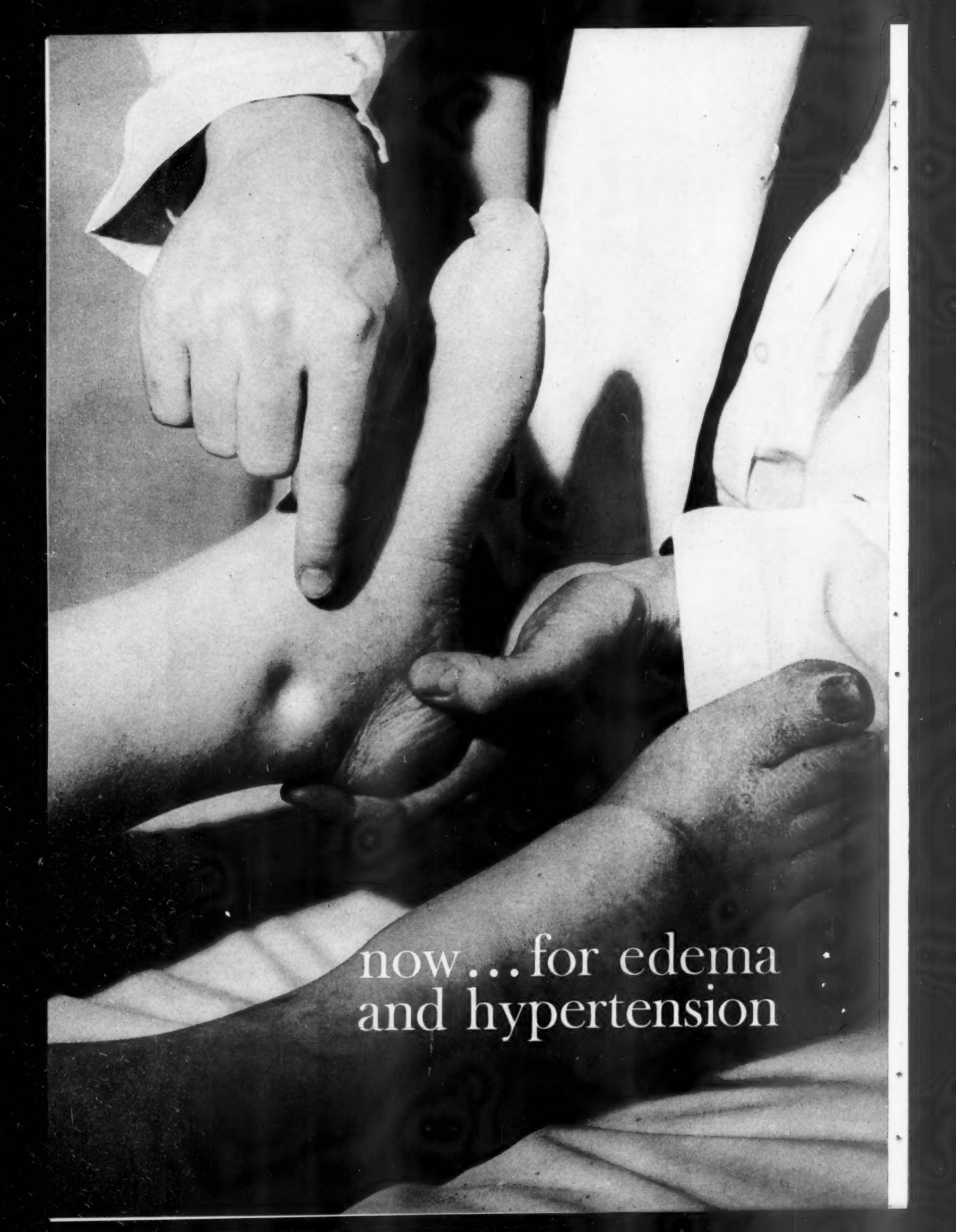


**Dosage:** the usual intra-articular, intra-bursal or soft tissue dose ranges from 20 to 30 mg. depending on location and extent of pathology.

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and hypertension

*See case history of this patient  
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lowest blood-pressure levels  
yet achieved with oral  
diuretic-antihypertensive  
therapy...

**Esidrix**<sup>T.M.</sup>  
(hydrochlorothiazide CIBA)



2/2683MK-1





*Record of patient with congestive failure,  
treated at a leading Philadelphia hospital.  
Photos used with permission of the patient.*

marked pitting  
edema (4+)  
cleared in 4 days  
with Esidrix

**ESIDRIX IS 10 TO 15 TIMES MORE ACTIVE THAN CHLOROTHIAZIDE**

**INDICATED IN . . . congestive heart  
failure • hypertension • hypertensive  
vascular disease • premenstrual edema  
• toxemia of pregnancy • edema of  
pregnancy • steroid-induced edema  
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**DOSAGE:** Esidrix is administered orally in an average dose of 75 to 100 mg. daily, with a range of 25 to 200 mg. A single dose may be given in the morning or tablets may be administered 2 or 3 times a day.

**SUPPLIED:** Tablets, 25 mg. (pink, scored); bottles of 100 and 1000. Tablets, 50 mg. (yellow, scored); bottles of 100 and 1000.

**C I B A**  
SUMMIT N.Y.



L.S., 81-year-old patient with complaint of painless hematuria admitted to hospital on 3/3/59. Past history included congestive heart failure of 15 years' duration. **Clinically significant symptoms:** expiratory wheezes over entire chest; bilateral coarse rales of both bases; slight abdominal distention (without evidence of ascites); palpable liver 2-3 fingerbreadths below rib cage; bilateral pitting edema (4+) of pretibial and ankle area. **Admission diagnosis:** hematuria of unknown origin; arteriosclerotic cardiovascular disease; poorly compensated heart failure; chronic pulmonary fibrosis with pulmonary insufficiency.



Patient was put on regimen of bed rest, moderate salt restriction, digitalis and pulmonary decongestants. When ankle edema, hepatic congestion and rales failed to clear by 3/6, Esidrix 50 mg. b.i.d. was ordered. By 3/8 L.S. had lost 3 pounds. Rales decreased; there was 1+ pitting edema of ankle area only. He felt more comfortable, was able to enjoy reading newspapers and magazines in bed.



By 3/11, patient's weight had dropped 2 more pounds. Ankle edema and lung rales were gone. Patient tolerated cystoscopy and fulguration of a small bleeding polyp in his bladder on 3/12 very well. Ambulatory on the 4th day of Esidrix therapy, L.S. visited his neighbors down the hall, played checkers with another patient. On 3/14 he was discharged.

Patient L.S. Date	3/4	3/5	3/6	3/7	3/8	3/9	3/10	3/11	3/12	3/13
Urinary Output (ml.)	840	690	960	2140	1230	660	1220	1350	--	--
Weight (lbs.)	139	--	--	--	136	--	--	134	--	--
Esidrix Dosage (mg./day)	0	0	50	100	100	100	100	100	50	100

# Esidrix<sup>T.M.</sup>

(hydrochlorothiazide CIBA)

- relieves edema in many patients refractory to other diuretics<sup>1</sup>
- often produces greater weight loss than parenteral mercurials or chlorothiazide<sup>2</sup>
- provides a greater average reduction in blood pressure than chlorothiazide<sup>3</sup>
- is exceptionally safe . . . reduces the likelihood of electrolyte imbalance

1. Brest, A. N., and Likoff, W.: Am. J. Cardiol. 3:144 (Feb.) 1959. 2. Clark, G. M.: Clinical report to CIBA.  
3. Dennis, E. W.: Clinical report to CIBA.

## DIARRHEAS! DYSENTERIES●

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Adequate dosage is essential. It is the galacturonic acid intake (2 to 4 grams of Pectin N.F. per day) that determines the effectiveness. In many instances the use of relatively inert adsorbent fillers has limited the amount of the therapeutic detoxicant, PECTIN, in the formulation to an inadequate dosage.

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Increases bulk and fluid retention of upper intestinal contents and imparts a smooth, gelatinous consistency • Lubricates the intestinal wall • Promotes normal peristalsis without mechanical irritation • Reduces intestinal pH • Inhibits growth of many putrefactive and otherwise undesirable microorganisms in the intestines without affecting normal flora • Promotes assimilation of essential nutrients • Helps to conjugate and eliminate toxins • Reduces toxic side effects of therapeutic agents.

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## HIGHLIGHTS FROM THE A.M.A. COUNCIL ON DRUGS REPORT ON TRIAMCINOLONE

*J.A.M.A.* 169:257 (January 17) 1959.

"It [triamcinolone] has an anti-inflammatory potency greater than an equal amount of prednisolone; i.e., comparable suppressive effects may usually be achieved with lower doses of triamcinolone than with prednisolone."

"Triamcinolone lacks the sodium-retaining and edema-producing effects of most other glucocorticoids. During the first several days of administration, it may cause a loss of sodium from the body; an initial mild diuretic action is frequently observed, whether the patient is frankly edematous or not. This is in contrast to the definite sodium-retaining and fluid-retaining properties of cortisone and hydrocortisone and to a much lesser extent with prednisone and prednisolone."

"Except in exceedingly large doses, triamcinolone apparently has no consistent effect on potassium excretion. Hence, neither sodium restriction nor potassium supplementation is ordinarily required during therapy with this agent."

"As with other glucocorticoids, the long-term administration of triamcinolone results in definite catabolic effects, as indicated by impairment of carbohydrate utilization and negative protein and calcium balance. This catabolic effect, coupled with a lack of appetite stimulation which is apparently peculiar to triamcinolone, may produce weight loss that might be undesirable in some patients treated for long periods of time."

"...the voracious appetite, with weight gain and euphoria, characteristic of other steroids, is not seen with administration of triamcinolone."

"Triamcinolone has been used for the management of a wide variety of clinical conditions usually considered amenable to systemic steroid therapy. These have included rheumatoid arthritis and other collagen diseases, allergic and dermatological disorders, certain leukemias and malignant lymphomas, the nephrotic syndrome, pulmonary emphysema and fibrosis, acute bursitis, rheumatic fever, and certain blood dyscrasias. Although clinical experience with the drug in some of the foregoing conditions is not extensive, the many similarities in action between triamcinolone and other potent glucocorticoids would indicate a usefulness for triamcinolone akin to that of other agents of this class."

"There is some evidence that triamcinolone is more effective at a smaller dosage than are other steroids in controlling both the skin and joint lesions in psoriasis, whether or not complicated by arthropathy."

"Triamcinolone appears to compare favorably with other steroids for use in those situations in which edema and sodium retention have been complicating problems."

"It [triamcinolone] may also be the steroid of choice for patients in whom psychic stimulation, euphoria, voracious appetite, and weight gain should be avoided."

"...the drug [triamcinolone] does produce the other side effects and untoward reactions common to the glucocorticoids. At therapeutically equivalent doses, the frequency and severity of clinical manifestations of hyperadrenalism — rounding of the face, fat deposition, and hirsutism — are essentially the same. Likewise, there is little indication that the relative incidence of osteoporosis is materially decreased after the long-term use of the drug."

"Triamcinolone apparently does not cause the euphoria sometimes seen with other steroids, and the occurrence of mental depressions is uncommon."

"Current evidence suggests that the drug [triamcinolone] may not produce as high an incidence of peptic ulcer as do other steroids."

"Cutaneous erythema seems to be a side effect peculiar to triamcinolone."

"The usual contraindications and precautions of glucocorticoid therapy should be followed in the use of triamcinolone, keeping in mind that prolonged therapy with this drug will suppress the function of the patient's own adrenals by interfering with the pituitary-adrenal axis."

# Aristocort<sup>®</sup>

Triamcinolone LEDERLE

Supplied: 1 mg. scored tablets (yellow)  
2 mg. scored tablets (pink)  
4 mg. scored tablets (white)



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# The Biological Effectiveness of Oatmeal Protein

The pattern of essential amino acids in food protein is the criterion of quality by which biological effectiveness is measured. The efficient conversion of ingested protein to tissue protein depends on the concomitant presence of all needed amino acids in proper amounts and proportions at the site of biosynthesis.

Oatmeal exceeds all other whole-grain cereals in the amount of protein it provides. The quality of its protein is good—the distribution pattern of essential amino acids of the protein afforded by the oatmeal-and-milk serving resembles remarkably closely the pattern required by man.

## Comparison of Pattern of Essential Amino Acids in Quaker Oats Breakfast Dish\* with Pattern of Essential Amino Acids Required by Male Adults

(Values on Basis of Tryptophan as Unity)

	Tryptophan	Phenylalanine	Lysine	Threonine	Valine	Methionine	Leucine	Isoleucine
Essential Amino Acids Pattern in Quaker Oats Breakfast Dish* (1)	1.0	3.8	4.2	2.9	4.7	1.4	6.4	4.3
Essential Amino Acids Pattern Required by Male Adults (2)	1.0	4.4	3.2	2.0	3.2	4.4	4.4	2.8

\*Prepared from 1 oz. Quaker Oatmeal (dry) and 4 fl. oz. whole milk.

(1) Estimated from values in "Amino Acid Content of Foods", Home Economics Research Report No. 4, U.S. Dept. Agr., 1957, pp. 48, 58.  
(Quaker Oats protein = 16.7%)

(2) Staff Report: "Rose Reports Human Amino Acid Requirements", Chem. Eng. News 27:1364 (1949).

Quaker Oats and Mother's Oats, the two brands of oatmeal offered by The Quaker Oats Company, are identical. Both brands are available in the Quick (cooks in one minute) and the Old-Fashioned varieties which are of equal nutrient value.

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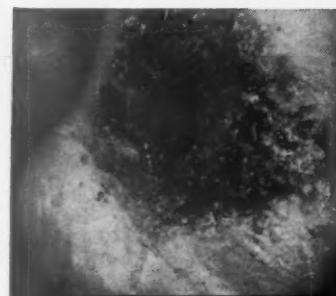
References: 1. Shelmire, J.B., Jr.: Monographs on Therapy 3:164 (Nov.) 1958. • 2. Nix, T.E., Jr., and Derbes, V.J.: Monographs on Therapy 3:123 (Nov.) 1958. • 3. Robinson, R.C.V.: Bull. School of Med., U. Maryland 43:54 (July) 1958. • 4. Sternberg, T.H.: Newcomer, V.D., and Reisner, R.M.: Monographs on Therapy 3:115 (Nov.) 1958. • 5. Clark, R.F., and Hallett, J.J.: Monographs on Therapy, 3:153 (Nov.) 1958. • 6. Smith J.G., Jr.; Zawisza, R.J., and Blank, H.: Monographs on Therapy, 3:111 (Nov.) 1958. • 7. Monographs on Therapy, 3:137 (Nov.) 1958. • 8. Howell, C.M., Jr.: North Carolina M.J. 19:449 (Oct.) 1958. • 9. Bereston, E.S.: South. M.J. 50:547 (April) 1957. And whatever the topical corticoid need, a suitable Squibb formulation is available — Kenalog-S Lotion — 7½ cc. plastic squeeze bottles. Each cc. supplies 1.0 mg. (0.1%) triamcinolone acetonide, 2.5 mg. neomycin base and 0.25 mg. gramicidin. Kenalog Cream, 0.1% — 5 Gm. and 15 Gm. tubes. Kenalog Lotion, 0.1% — 15 cc. plastic squeeze bottles. Kenalog Ointment, 0.1% — 5 Gm. and 15 Gm. tubes.



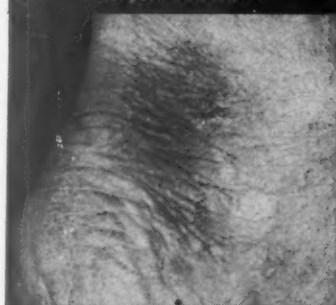
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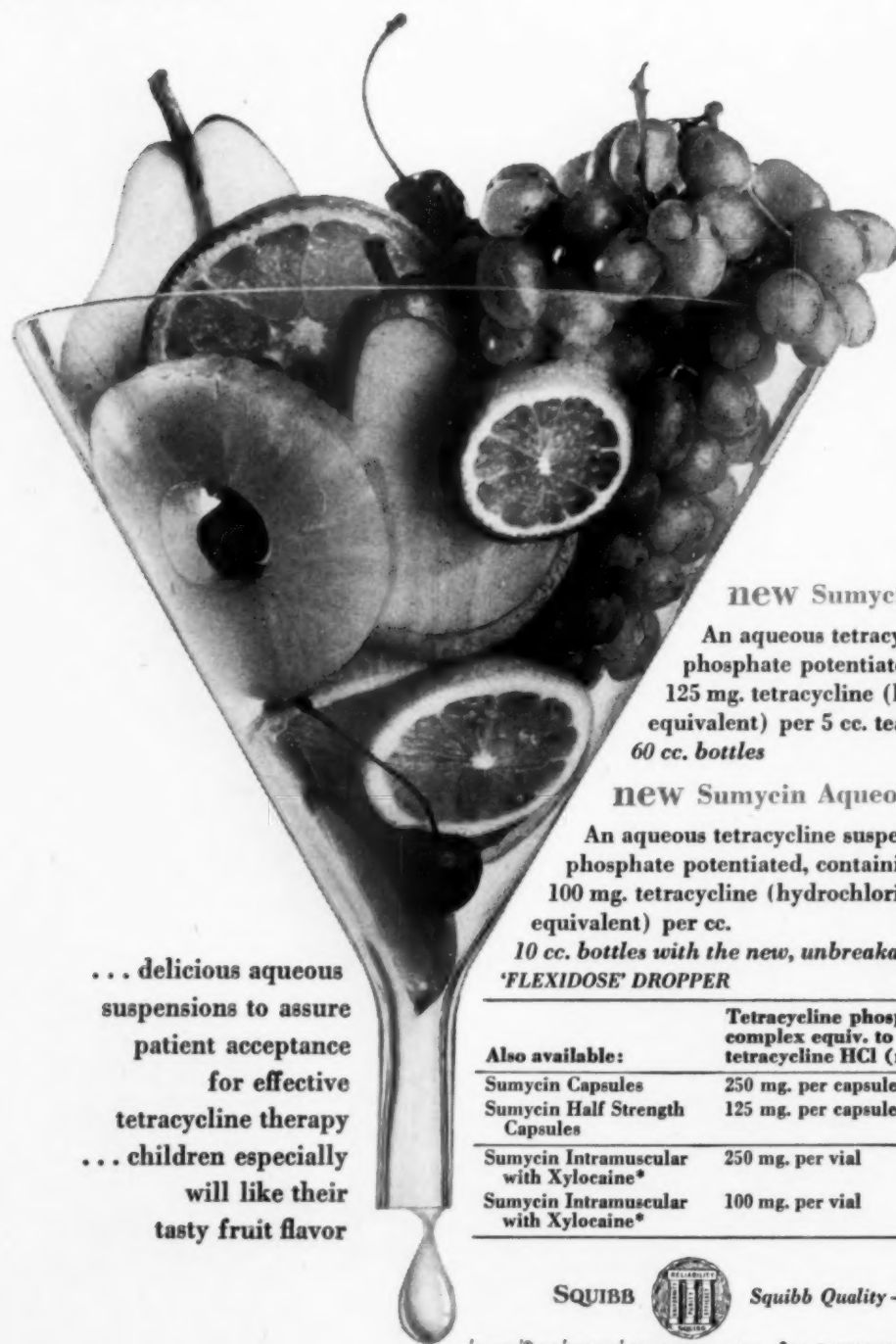


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hyperemotivity

ENARAX combines a new long-acting anticholinergic (antisecretory-antispasmodic<sup>1</sup>) with the proven antisecretory-tranquilizer<sup>2-6</sup> (ATARAX<sup>®</sup>) to relieve pain, spasm, hyperacidity and disease-induced tension. With this unique anticholinergic, just two tablets daily proved clinically effective in 428 out of 490 patients with a wide variety of gastrointestinal disorders.<sup>1,2,7</sup>

Selective postganglionic action on the G.I. tract minimizes side effects. Of 512 patients treated to date, reactions were usually mild, transient and easily reversed. Mouth dryness, blurring of vision, dizziness, urinary hesitancy either disappeared with continued use or were controlled by reducing the dosage.

# ENA



# ENARAX

one tablet at breakfast  
one tablet at bedtime

full-time relief in

peptic ulcer	gastritis	duodenitis
functional bowel syndrome	gastroenteritis	hiatus hernia (symptomatic)
ulcerative colitis	pylorospasm	genitourinary spasm
biliary tract dysfunctions	cardiospasm	dysmenorrhea

Each ENARAX tablet contains:

Oxyphencyclimine HCl... 10 mg.,

Hydroxyzine HCl (ATARAX®)... 25 mg.

*Dosage:* One-half to one tablet twice daily — preferably in the morning and before retiring. The maintenance dose should be adjusted according to therapeutic response. Use with caution in patients with prostatic hypertrophy or glaucoma.

*Supplied:* In bottles of 60 black-and-white scored tablets.

*References:* 1. McHardy, G., et al.: Paper presented at Postgraduate Course in Gastroenterology, University of California School of Medicine, San Francisco, California, January 27, 1958. 2. Strub, I. H., and Carballo, A.: To be published. 3. Schuller, E.: *Gaz. des Hôpitaux* 10:391 (Apr. 10) 1957. 4. Farah, L.: *Internat. Rec. Med.* 169:379 (June) 1956. 5. La Barre, J.: *Compt. rend. Soc. Biol. (Paris)* 150:1807 (Oct.) 1956. 6. Harrison, J. W. E., et al.: Paper presented at the 4th Pan-American Congress of Pharmacy and Biochemistry, Washington, D. C., November 3-9, 1957. 7. Data in Roerig Medical Department files.



New York 17, N. Y.

Division, Chas. Pfizer & Co., Inc.

Science for the World's Well-Being

# RAX



A SENTRY FOR THE G.I. TRACT

**FRIDAY**

1959	MARCH						1959
SUN	MON	TUE	WED	THU	FRI	SAT	
1	2	3	4	5	6	7	
8	9	10	11	12	13	14	
15	16	17	18	19	20	21	
22	23	24	25	26	27	28	
29	30	31					

**27**

**MAR.  
1959**

**sulfa**

**the tablet he took yesterday morning...**



# SATURDAY

1959 FEBRUARY 1959						
S	M	T	W	T	F	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28

28  
MAR.  
1959

1959 APRIL 1959						
S	M	T	W	T	F	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30		

is still working this morning

# Midicel<sup>®</sup>

(sulfamethoxypyridazine, Parke-Davis)

maintains effective  
sulfa levels  
for 24 hours  
with a single tablet

MIDICEL differs from older sulfonamides because it affords *all* these clinical advantages: *1 tablet-a-day schedule*—greater convenience and economy for patients • *rapid effect*—prompt absorption • *prolonged action*—effective plasma and tissue concentrations sustained day and night with 1 tablet daily • *wide antibacterial spectrum*—effective in urinary tract infections, upper respiratory infections, bacillary dysenteries, and surgical and soft tissue infections, due to sulfonamide-sensitive organisms • *well tolerated*—low dosage and high solubility minimize possibility of crystalluria.

*adult dosage:* Initial (first day)—2 tablets (1 Gm.) for mild or moderate infections, or 4 tablets (2 Gm.) for severe infections. Maintenance—1 tablet (0.5 Gm.) daily.

*children's dosage:* According to weight.

See literature for details of dosage and administration.

*available:* Quarter-scored tablets of 0.5 Gm., bottles of 24, 100, and 1,000.



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72359



In hypoprothrombinemia

# STOP BLEEDING BEFORE IT BEGINS

## PROPHYLACTICALLY

and

## THERAPEUTICALLY

**Before delivery** KONAKION prevents neonatal hemorrhage, rapidly establishes physiologic prothrombin levels in the newborn.

**Before surgery** KONAKION minimizes the risk of hemorrhage by normalizing prothrombin time, especially important in biliary procedures.

### KONAKION for

- excessive hypoprothrombinemia due to anticoagulant therapy
- inadequate intake or utilization of vitamin K
- neonatal hemorrhage
- g.i. disorders with prolonged diarrhea

KONAKION—a potent synthetic vitamin K<sub>1</sub> with a wide margin of safety and rapid rate of absorption unequaled by other vitamin K preparations. Convenient low-dosage forms for *oral*, *intramuscular*, or *intravenous* administration. The parenteral form is an *exceptionally fine aqueous dispersion*, compatible with most I.V. vehicles, and packaged for economical one-time use.

# NEW KONAKION®

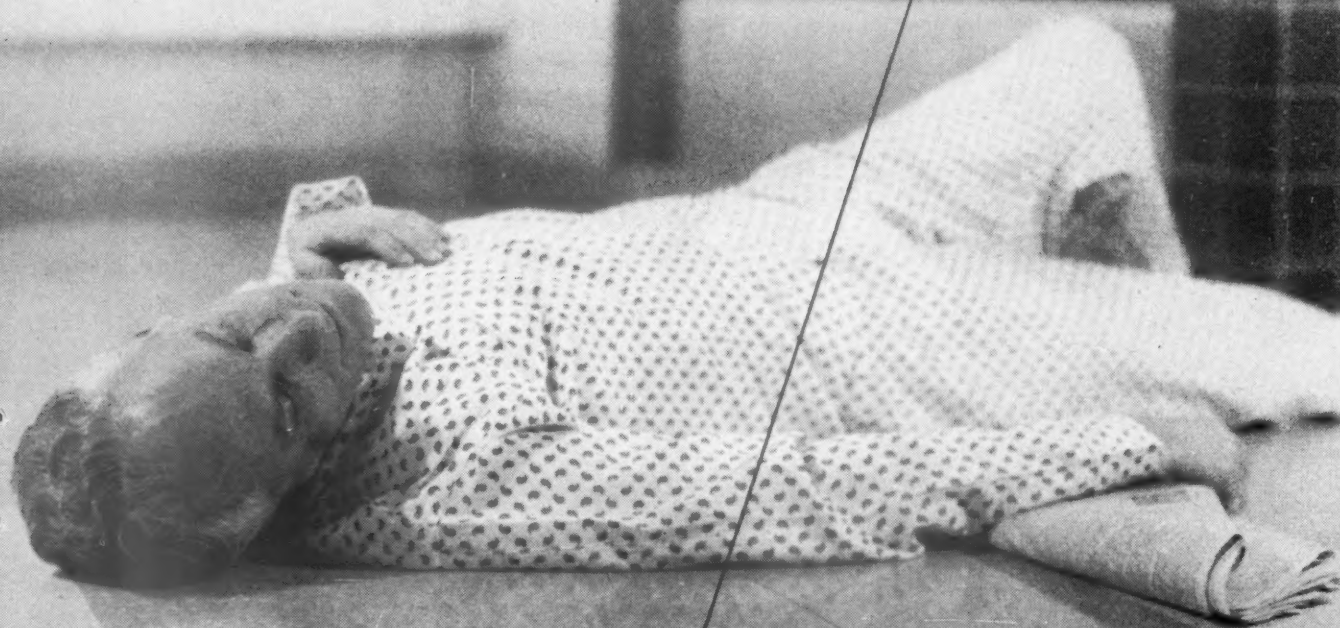
Capsules—5 mg; Ampuls—1 mg/0.5 cc, 10 mg/1.0 cc, 25 mg/2.5 cc

brand of vitamin K<sub>1</sub>

ROCHE LABORATORIES • Division of Hoffmann-La Roche Inc • Nutley 10, New Jersey



*might Colace  
have prevented this?*



As Dennison<sup>1</sup> reported, "It is our considered opinion that the relief from straining at the stool is, in many instances, life-saving." And, further, "In the handling of bowel problems in cardiac patients, the properties of dioctyl sodium sulfosuccinate closely approach those required of an ideal agent."

**Colace<sup>®</sup>**

*Dioctyl sodium sulfosuccinate, Mead Johnson*

prevents hard, difficult-to-pass stools . . . without laxative action.

available in 3 convenient dosage forms:

**capsules** (50 and 100 mg.) . . . for adults and older children

**syrup** . . . for children and adults

**liquid (drops)** . . . for infants and children



**Mead Johnson**  
*Symbol of service in medicine*

<sup>1</sup> Dennison, A.D., Jr.: Am. J. Cardiol. 400-403 (March) 1958.



in arthritis

# Butazolidin®

(phenylbutazone Geigy)

tablets • alka capsules

potent • nonhormonal • anti-inflammatory agent

BUTAZOLIDIN tablets or the Alka capsules are **equally effective but individually adaptable in a wide range of arthritic disorders.**

Recent clinical reports continue to justify the selection of Butazolidin for rapid relief of pain, increased mobility, and early resolution of inflammation.

**Gouty Arthritis:** "...95 per cent of patients experienced a satisfactory response..."<sup>1</sup>

**Rheumatoid Arthritis:** In "A total of 215 cases...over half, 50.7 per cent showed at least major improvement,

with 21.8 per cent showing minor improvement..."<sup>2</sup> **Osteoarthritis:** 301 cases showed "...a total of 44.5 per cent with complete remission or major improvement. Of the remainder, 28.2 per cent showed minor improvement..."<sup>3</sup> **Spondylitis:** All patients "...experienced initial major improvement that was maintained throughout the period of medication."<sup>3</sup> **Painful Shoulder Syndrome:** Response of 70 patients with various forms showed "...8.6 per cent complete remissions, 47.1 per cent major improvement, 20.0 per cent minor improvement..."<sup>2</sup>

**References:** 1. Graham, W.: *Canad. M. A. J.* 79:634 (Oct. 15) 1958. 2. Robins, H. M.; Lockie, L. M.; Norcross, B.; Latona, S., and Riordan, D. J.: *Am. Pract. Digest Treat.* 8:1758, 1957. 3. Kuzell, W. C.; Schaffarzick, R. W.; Naugler, W. E., and Champlin, B. M.: *New England J. Med.* 256:388, 1957.

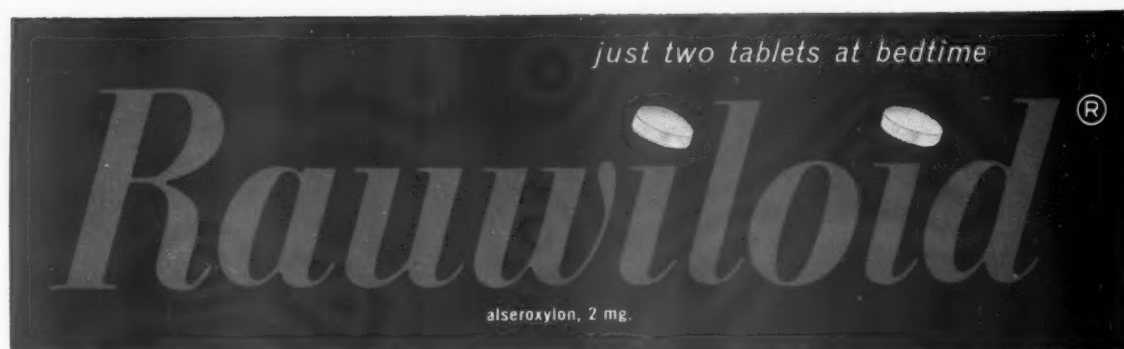
**Availability** BUTAZOLIDIN® (phenylbutazone Geigy): Red coated tablets of 100 mg. BUTAZOLIDIN® Alka: Capsules containing BUTAZOLIDIN® (phenylbutazone Geigy), 100 mg.; dried aluminum hydroxide gel, 100 mg.; magnesium trisilicate 150 mg.; homatropine methylbromide, 1.25 mg.

**geigy**  
ARDSLEY, NEW YORK

GEIGY



# Classic Treatment in Hypertension\*



When more potent drugs are needed, prescribe one of the convenient single-tablet combinations

**Rauwiloid® + Veriloid®**

alseroxylon 1 mg. and alkavervir 3 mg.

or

**Rauwiloid® + Hexamethonium**

alseroxylon 1 mg. and hexamethonium chloride dihydrate 250 mg.

Many patients with severe hypertension can be maintained on Rauwiloid alone after desired blood pressure levels are reached with combination medication.

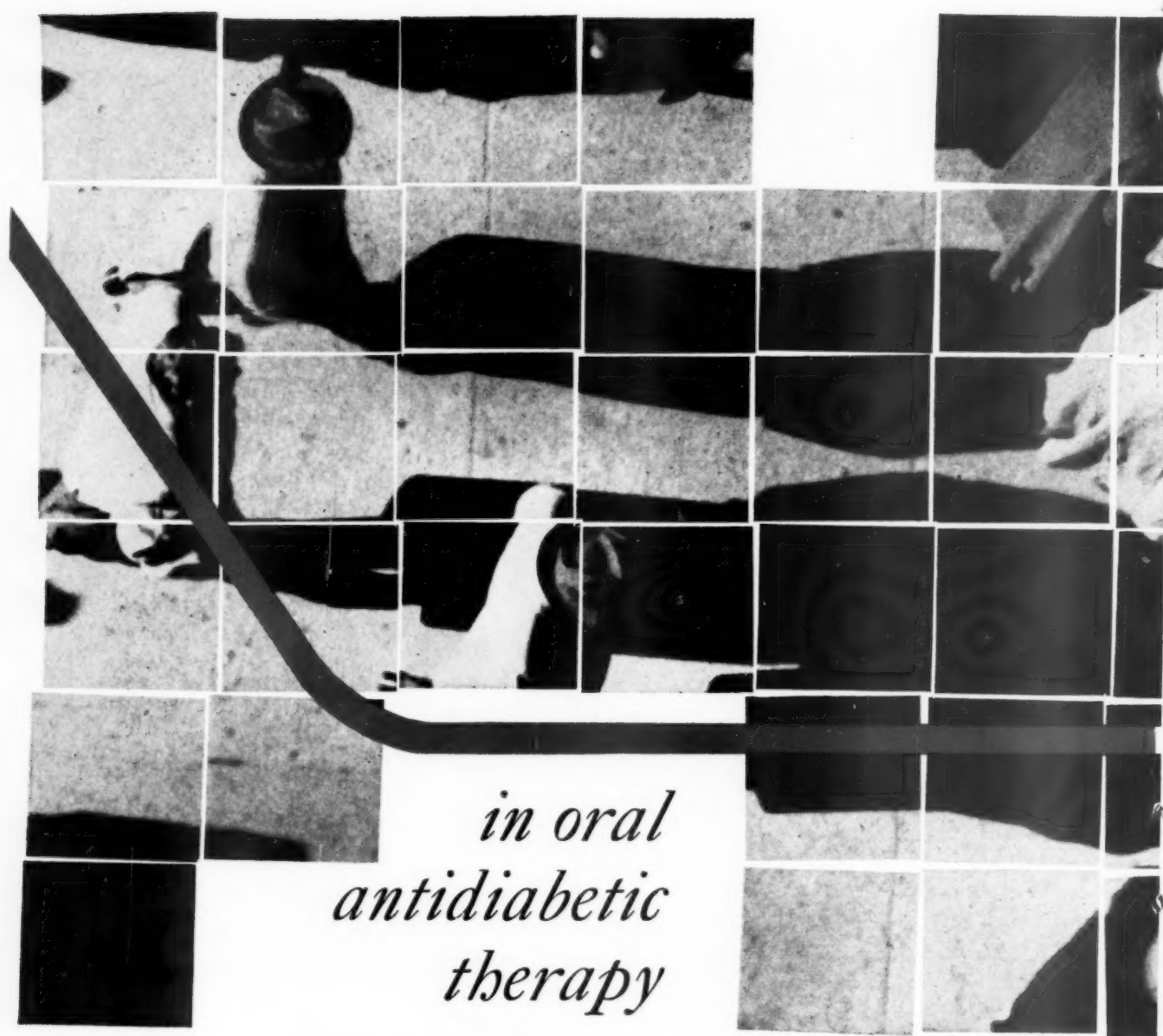
## \*Because

RAUWILOID provides effective Rauwolfia action virtually free from serious side effects ... the smooth therapeutic efficacy of Rauwiloid is associated with a lower incidence of certain unwanted side effects than is reserpine... and with a lower incidence of depression. Tolerance does not develop.

RAUWILOID can be initial therapy for most hypertensive patients... Dosage adjustment is rarely a problem.



Northridge, California



*in oral  
antidiabetic  
therapy*

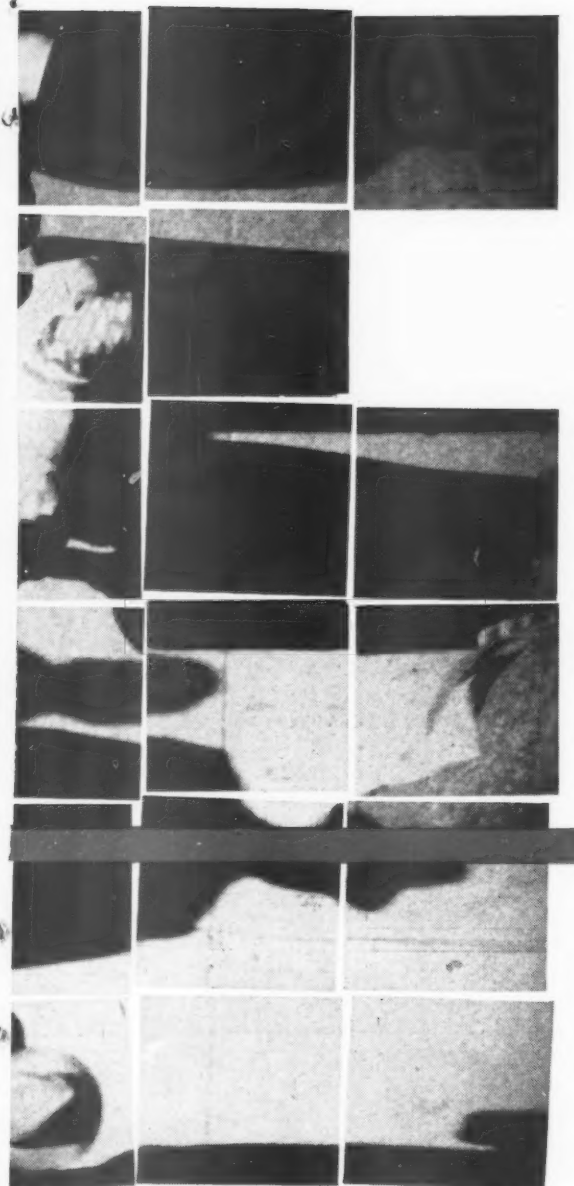
**DIABINESE<sup>®</sup>**

brand of chlorpropamide

*provides the POTENCY ESSENTIAL for  
predictable, precise response*

Science for the world's well-being





## Economical once-a-day dosage

*Supplied: Tablets, white, scored,  
250 mg., bottles of 60 and 250;  
100 mg. bottles of 100.*

1. Sugar, S., et al.: M. Ann. District of Columbia 27:445, 1958. 2. O'Driscoll, B. J.: J. Irish M. A. 43:323, 1958. 3. Greenhouse, B.: In Conference on Diabinese and Diabetes Mellitus, New York Acad. Sc., Sept. 25-27, 1958, New York, N.Y. 4. Sheppe, W. M.: West Virginia M. J. 54:467, 1958. 5. Schumacher, O. P., et al.: Cleveland Clinic Quart. 26:12, Jan., 1959.

PFIZER LABORATORIES, Brooklyn 6, N. Y.  
Division, Chas. Pfizer & Co., Inc.

## EFFECTIVENESS

**DIABINESE** increases the opportunity of success and minimizes the danger of therapeutic failure. It has the necessary *potency* to assure effective reduction of blood sugar in most maturity-onset diabetics—even in patients who have failed to respond to other oral therapy.<sup>1</sup>

**DIABINESE** is eliminated *gradually* as the active substance, thus permitting "more even reduction of the blood sugar..."<sup>2</sup> Its effect is "devoid of marked blood sugar fluctuations and wide metabolic excursions"<sup>3</sup> observed with less potent, readily metabolized medication requiring multiple dosage.

## SAFETY

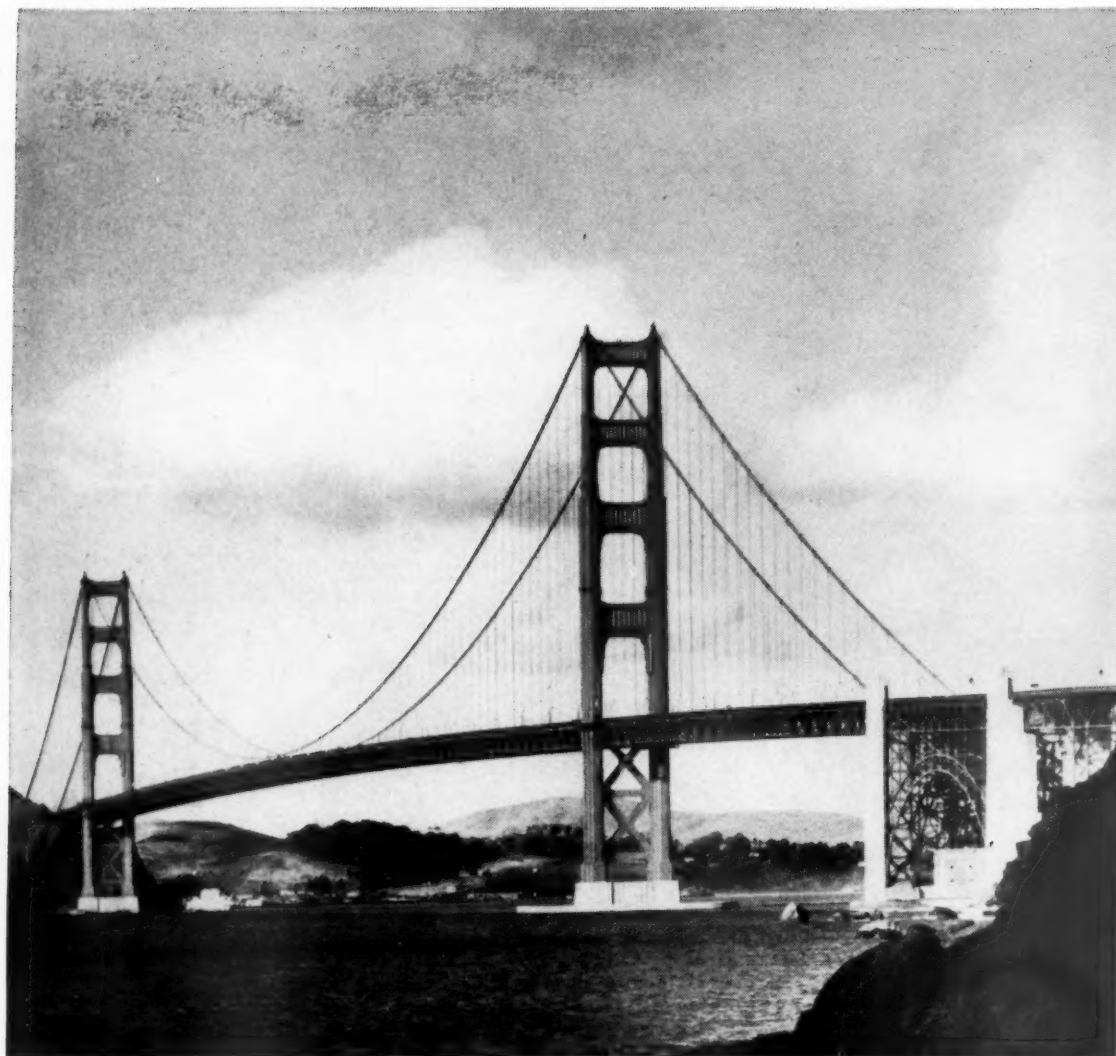
**DIABINESE** is "well tolerated with minimal side effects in the therapeutic range of 100 to 500 mg."<sup>3</sup> Its striking effectiveness and "almost complete absence of unfavorable side effects" have led to the prediction that "Diabinese will eventually prove to be the drug of choice in the sulfonylurea group."<sup>4</sup>

## ECONOMY AND CONVENIENCE

**DIABINESE** has the necessary *duration* of effect to permit convenient once-a-day dosage. Moreover, the average dose of **DIABINESE** (285 mg. per day)<sup>5</sup> means a substantial reduction in cost to your patients.

# DIABINESE®





Majestically simple in design, the Golden Gate Bridge is the longest single span suspension bridge in the world

## **T**HINGS THAT ENDURE

Good things endure... a work of art, a literary classic, a proud bridge... a dependable pharmaceutical. Such is **Desitin Ointment**. For over 35 years Desitin Ointment has endured as an incomparable, safe way to prevent and clear up diaper rash... and as a soothing, healing application in wounds, burns, external ulcers and other skin injuries.

Desitin®

**DESITIN CHEMICAL COMPANY** Providence 4, R. I.

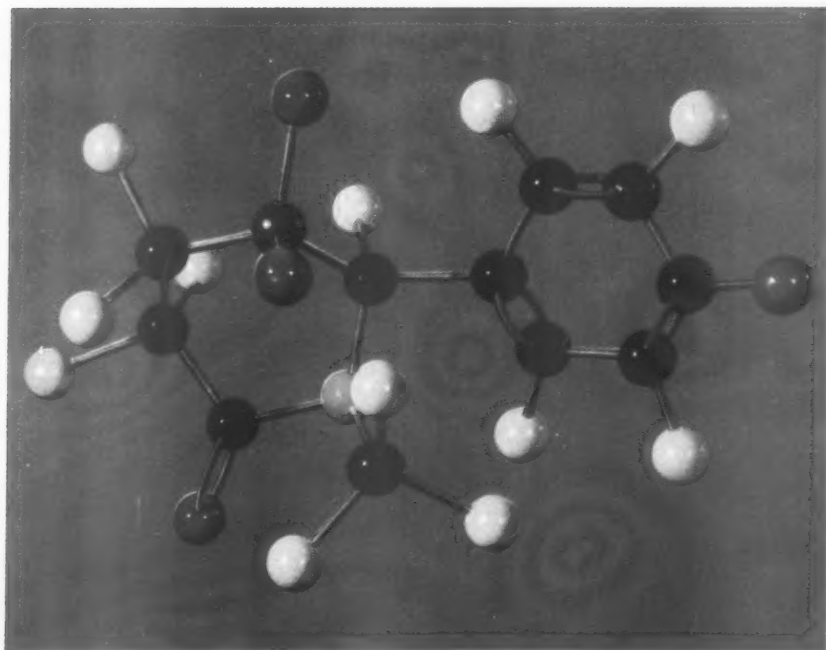
2½ minutes of your time reading about  
Trancopal may change your prescription  
habits when treating musculoskeletal and  
psychogenic disorders.

# *Trancopal*®

*the first true tranquilaxant\**

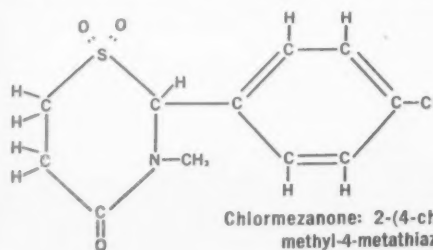
**Potent MUSCLE RELAXANT**  
**...Equally effective as a TRANQUILIZER**

\* **tran-qui-lax-ant** (tran'kwi-lak'sant) [ < L. *tranquillus*,  
quiet; L. *laxare*, to loosen, as the muscles ]



Trancopal, a major development of Winthrop research, is a new orally administered nonhypnotic central relaxant and tranquilizer. It relieves muscle spasm in a variety of musculoskeletal and neurologic conditions and also exerts a marked tranquilizing effect in anxiety and tension states.

Unrelated chemically to any other drug in current use, Trancopal offers a completely new major chemical contribution to therapeutics.



## *Thoroughly evaluated clinically...*

Clinical studies of 4092 patients by 105 physicians<sup>1</sup> have demonstrated that Trancopal often is effective when other drugs have failed. From these studies it is evident that Trancopal can provide more help for a greater number of tense, spastic, and/or emotionally upset patients than can any other chemotherapeutic agent in current use.

## **In musculoskeletal conditions<sup>1</sup>**

effective in **91%** of patients

### **INDICATIONS**

Low back pain (lumbago)	Neck pain (torticollis)
Bursitis	Rheumatoid arthritis
Osteoarthritis	Disk syndrome
Fibrositis	Joint disorders (ankle sprain,
Myositis	tennis elbow, etc.)
Postoperative myalgias	

By relieving muscle spasm and pain, Trancopal permits early and active purposeful exercise and physical therapy to accomplish maximal benefits for rapid recovery.

# ***Trancopal***

**Dosage:** One Caplet (100 mg.) orally three or four times daily. Relief of symptoms occurs in fifteen to thirty minutes and lasts from four to six hours.



In anxiety and tension states<sup>1</sup>

effective in **88%** of patients

### INDICATIONS

Anxiety and tension states

Dysmenorrhea

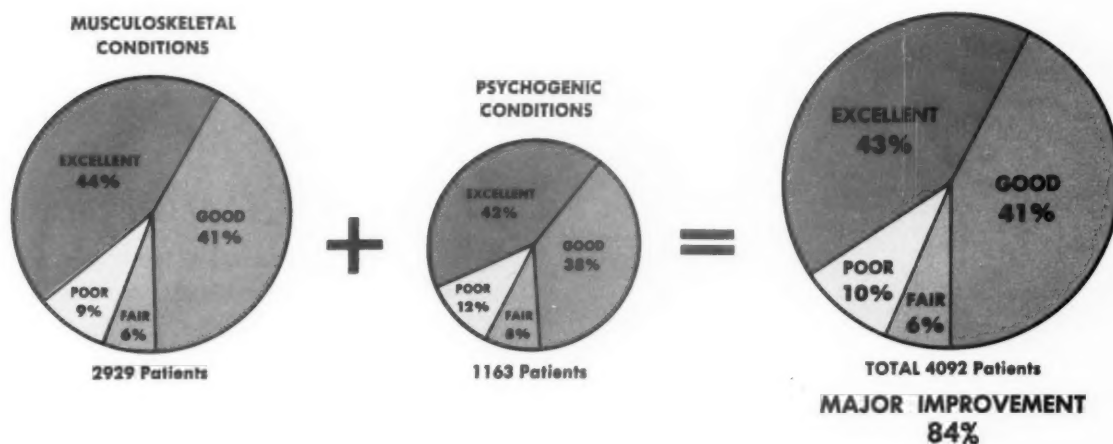
Premenstrual tension

Asthma

Emphysema

Angina pectoris

Because of its exceptional calmative property, Trancopal "...allows the patient to use his energies in a more productive manner in overcoming his basic problem."<sup>2</sup>



Of the total patients treated, Trancopal produced excellent results in 43 per cent, good results in 41 per cent, fair results in 6 per cent, and poor results in 10 per cent.

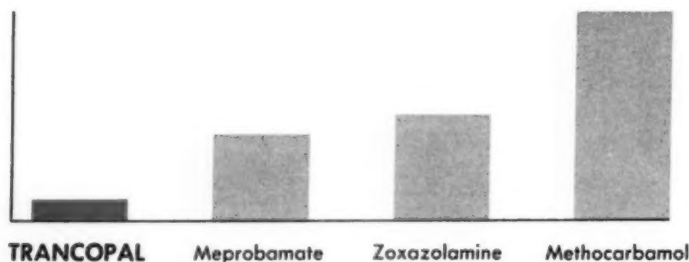
### *Better tolerated and safer than older drugs<sup>3</sup>*

With Trancopal there is no clouding of consciousness, no euphoria or depression. Even in high dosage, there is no perceptible soporific effect. Because it does not irritate gastric mucosa, it can be taken without regard to mealtimes. Administration does not hamper work — or play. There are no known contraindications. Blood pressure, pulse rate, respiration and digestive processes are unaffected by therapeutic dosage.

Toxicity is extremely low. And Trancopal has a lower incidence of side effects than has zoxazolamine, methocarbamol or meprobamate.

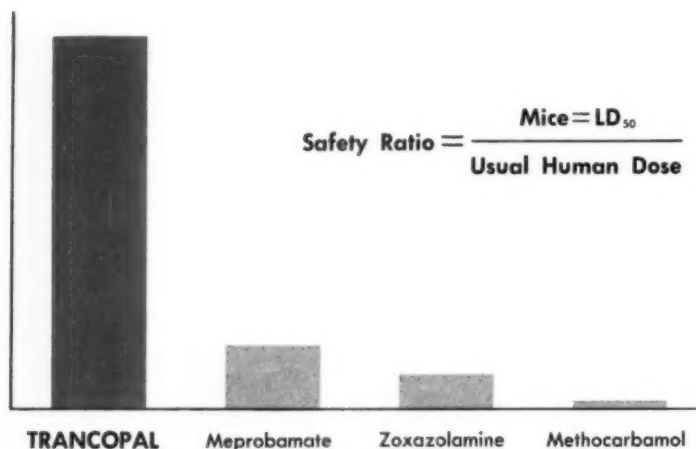
## Comparison with 3 widely used central relaxants

When compared with three widely used central relaxants for activity, safety and clinical effectiveness, Trancopal offers definite desirable advantages.



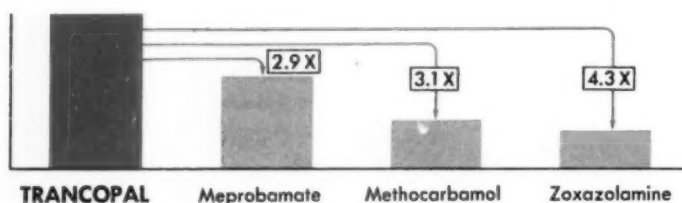
### *for activity*

In the usual human dose, Trancopal is four to ten times as potent per milligram.



### *for safety*

Comparative pharmacologic tests showed that Trancopal is up to thirteen times as safe or up to thirteen times less toxic. The measure of safety was the LD<sub>50</sub> in mice/usual human dose.



### *for clinical effectiveness*

A clinical comparison in low back pain, torticollis, bursitis and anxiety states showed that Trancopal is up to four times as effective. Each of the 40 patients received all four drugs in random rotation for several days. Although each of the four gave some relief, only the one providing the most effective relief was recorded.

**Supplied:** Trancopal Caplets® (scored) 100 mg., bottles of 100.

**References:** 1. Cooperative Study, Department of Medical Research, Winthrop Laboratories. • 2. Gans, S.E.: To be published. • 3. Lichtman, A.L.: Kentucky Acad. Gen. Pract. J. 4:28, Oct., 1958.

*the first true tranquilaxant*  
**Trancopal**

**Potent**  
**MUSCLE RELAXANT**  
*...Equally effective as a*  
**TRANQUILIZER**

*Winthrop*

Laboratories

New York 18, New York

Trancopal (brand of chlormezanone) and Caplets,  
 trademarks reg. U. S. Pat. Off.

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"PREMARIN" WITH MEPROBAMATE

# 200

## FOR PROVEN MENOPAUSAL BENEFITS

The vast majority of menopausal women, especially on the first visit, are nervous, apprehensive, and tense. PMB-200 or PMB-400 gives your patient the advantage of extra relief from anxiety and tension, particularly when the patient is "high strung," under prolonged emotional stress, or when psychogenic manifestations are acute. Proven menopausal benefits are confirmed by the wide clinical acceptance of "Premarin," specifically for the relief of hot flushes and other symptoms of estrogen deficiency, together with the well established tranquilizing efficacy of meprobamate.



Two potencies that will meet the needs of your patients: PMB-200 — Each tablet contains conjugated estrogens equine ("Premarin") 0.4 mg., and 200 mg. of meprobamate. When greater tranquilization is necessary you can prescribe PMB-400 — Each tablet contains conjugated estrogens equine ("Premarin") 0.4 mg., and 400 mg. of meprobamate. Both potencies are available in bottles of 60 and 500.

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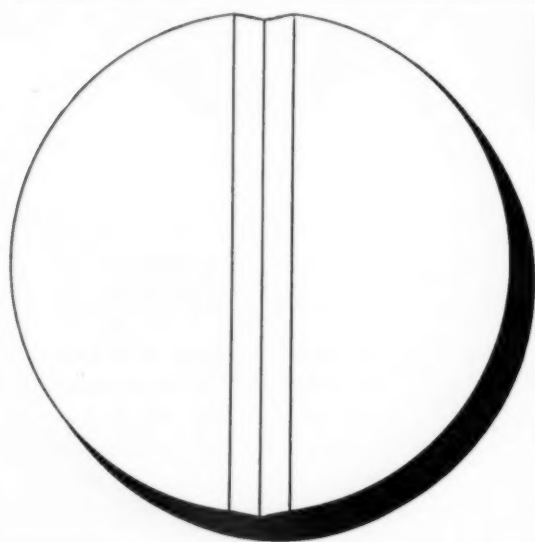
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AT LOW COST TO YOUR PATIENT



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Squibb 400,000 units Buffered Penicillin G Potassium Tablets

For the treatment of penicillin susceptible infections—  
ranging from mild to moderately severe—due to  
**hemolytic streptococcus / pneumococcus / staphylococcus /**  
and for the prevention of streptococcal infections where there is  
a history of rheumatic fever

Clinical effectiveness confirmed by millions of cases  
Specific in many common infections  
Daily dosage may be spaced without regard to mealtime  
Ease of administration with oral penicillin  
Economy for the patient

**SQUIBB**



Squibb Quality—the Priceless Ingredient

**new convenient oral tablets**  
PENTIDS '400,' each scored tablet contains 400,000 units of penicillin G potassium buffered, bottles of 12 and 100. Twice the unitage of Pentids 200,000 units.

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**PENTIDS FOR SYRUP**, 200,000 units of penicillin G potassium per teaspoonful (5 cc.), 12 dose bottles.

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**PENTIDS SOLUBLE TABLETS**, 200,000 units of penicillin G potassium per tablet, vials of 12 and bottles of 100.

**PENTIDS-SULFAS TABLETS**, 200,000 units of penicillin G potassium with 0.5 Gm. triple sulfas per tablet, bottles of 30, 100, and 500.

in all  
diarrheas

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EXPERIENCE

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regardless of  
etiology



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**Consensus:**  
**The preferred antidote**  
**for anticoagulant-induced**  
**hypoprothrombinemia**  
**is 'Mephyton' (vitamin K<sub>1</sub>).**





"...has a more prompt, more potent and more prolonged effect than the vitamin K analogues....Its reliability in treating undue hypoprothrombinemia from anticoagulant therapy is of particular importance. [Mephyton] can be depended on to reverse anticoagulant-induced hypoprothrombinemia to safe levels whether bleeding is only potential or actually has occurred."

Council on Drugs: New and Nonofficial Drugs, Philadelphia, J. B. Lippincott Co., 1958, p. 620.

"For correction of the anticoagulant effect of the coumarin compounds, vitamin K<sub>1</sub> is much more effective than are the water-soluble preparations of menadione."

Barker, N. W.: Fundamentals of anticoagulant therapy, Minn. Med. 41:252, April 1958.

For coumarin overdosage, "Vitamin K<sub>1</sub>, given intravenously, in an oil emulsion will act as soon as two hours after injection. It is the treatment of choice in such conditions."

Kupfer, H. G., and Kinne, D. R.: Anticoagulants, theoretical considerations and laboratory control, Virginia M. Monthly 85:230, May 1958.

"...I would strongly urge the use of vitamin K<sub>1</sub>...if an antidote is necessary for the hypoprothrombinemia produced by the coumarin anticoagulants or the indandiones."

Meyer, O. O.: Use of anticoagulants in the treatment of coronary artery disease, Postgrad. Med. 24:110, Aug. 1958.

**chemically identical with naturally-occurring vitamin K<sub>1</sub>**

# Mephyton<sup>®</sup>

**Vitamin K<sub>1</sub>**

**Dosage:** Orally, to modify anticoagulant effects: 5 to 10 mg. initially; 15 to 25 mg. for more vigorous action. Intravenously, for anticoagulant-induced bleeding emergencies, 10 to 50 mg.; may be repeated as indicated by prothrombin time response. (Some clinicians advise their patients to keep a supply of tablets on hand at all times; if gross bleeding occurs, the patients are instructed to take 10 mg. and phone the doctor.)

**Supplied:** Tablets, 5 mg.; bottles of 100. Emulsion, each 1-cc. ampul contains 50 mg.; boxes of 6 ampuls.



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INTRODUCING

**RUBRAMIN**

SQUIBB VITAMIN B<sub>12</sub> U.S.P. INJECTION

**PC**

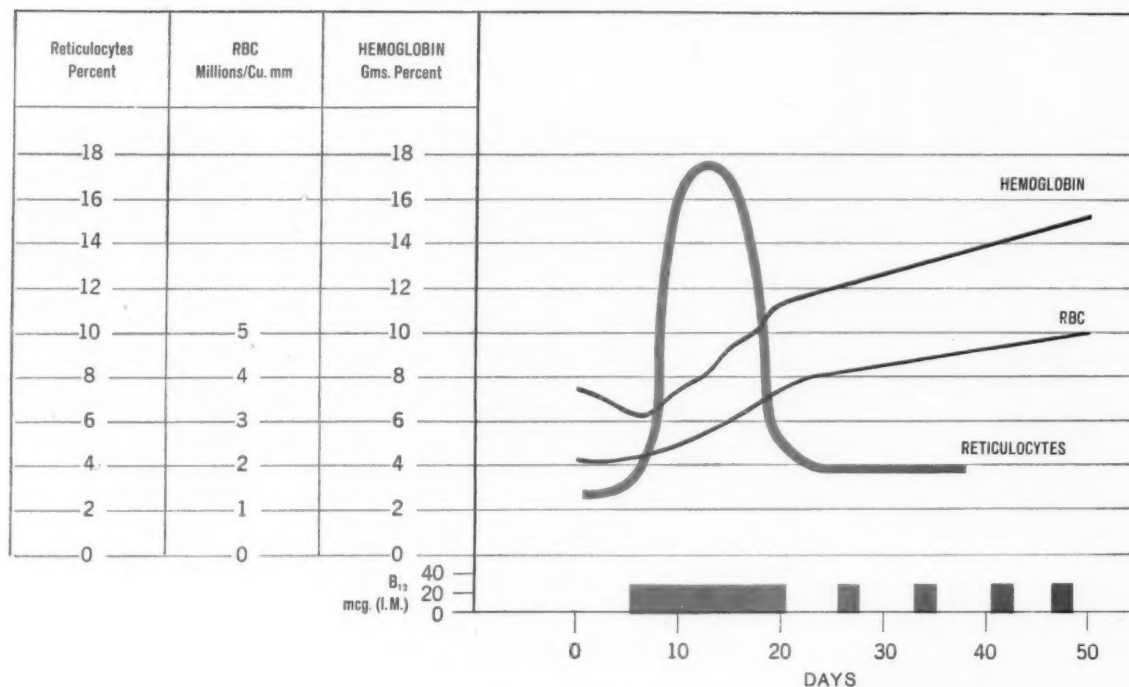
PURE CYANOCOBALAMIN INJECTION – CREATED AND PRODUCED  
BY SQUIBB – FOR THE MOST EXACTING STANDARDS OF INTRAVENOUS,  
INTRAMUSCULAR AND SUBCUTANEOUS ADMINISTRATION IN:

- *pernicious anemia*
- *severe nutritional macrocytic anemias*
- *severe nutritional neuropathies*
- *prevention of macrocytic anemia following  
partial or total gastrectomy*

and for the relief of pain in such conditions as:

trigeminal neuralgia; osteoarthritis; secondary burning paresthesias; herpes zoster; and  
neuroblastoma in children.

RUBRAMIN PC is highly effective whenever high doses of vitamin B<sub>12</sub> are required.



TYPICAL HEMATOPOIETIC RESPONSE WHEN RUBRAMIN P C IS GIVEN INTRAMUSCULARLY TO A PATIENT WITH MEGALOBlastic ANEMIA (schematic)

### highly potent and vital to metabolism

Vitamin B<sub>12</sub>—one of the most potent biological factors known—is vital to basic metabolic functions, to normal formation of red blood cells and other formed elements of the blood, and to the functional integrity of myelinated fibers in the spinal cord and brain, as well as to the healthy condition of gastric and oral mucosa.

### therapeutic agent of choice in pernicious anemia

Vitamin B<sub>12</sub> is the therapeutic agent of choice in pernicious anemia, is effective in certain megaloblastic macrocytic anemias, and contributes to recovery or clinical improvement in a variety of neurological, liver and skin disorders.

### non-toxic, remarkably free from side reactions

Despite its high level of activity, vitamin B<sub>12</sub> is non-toxic and remarkably free from side reactions. It has been well-tolerated even when administered in massive doses.

### potency confirmed by precise radioisotope measurement

The Radioisotope Tracer Method is now used routinely as an assay procedure in the production of RUBRAMIN, guaranteeing accurate label potency.

RUBRAMIN P C is now available in potencies for all your parenteral requirements: 30, 50 and 100 mcg. per cc., 10 cc. vials; 1000 mcg. per cc., 1 cc. and 10 cc. vials.

**SQUIBB**



*Squibb Quality—the Priceless Ingredient*

\*RUBRAMIN® IS A SQUIBB TRADEMARK



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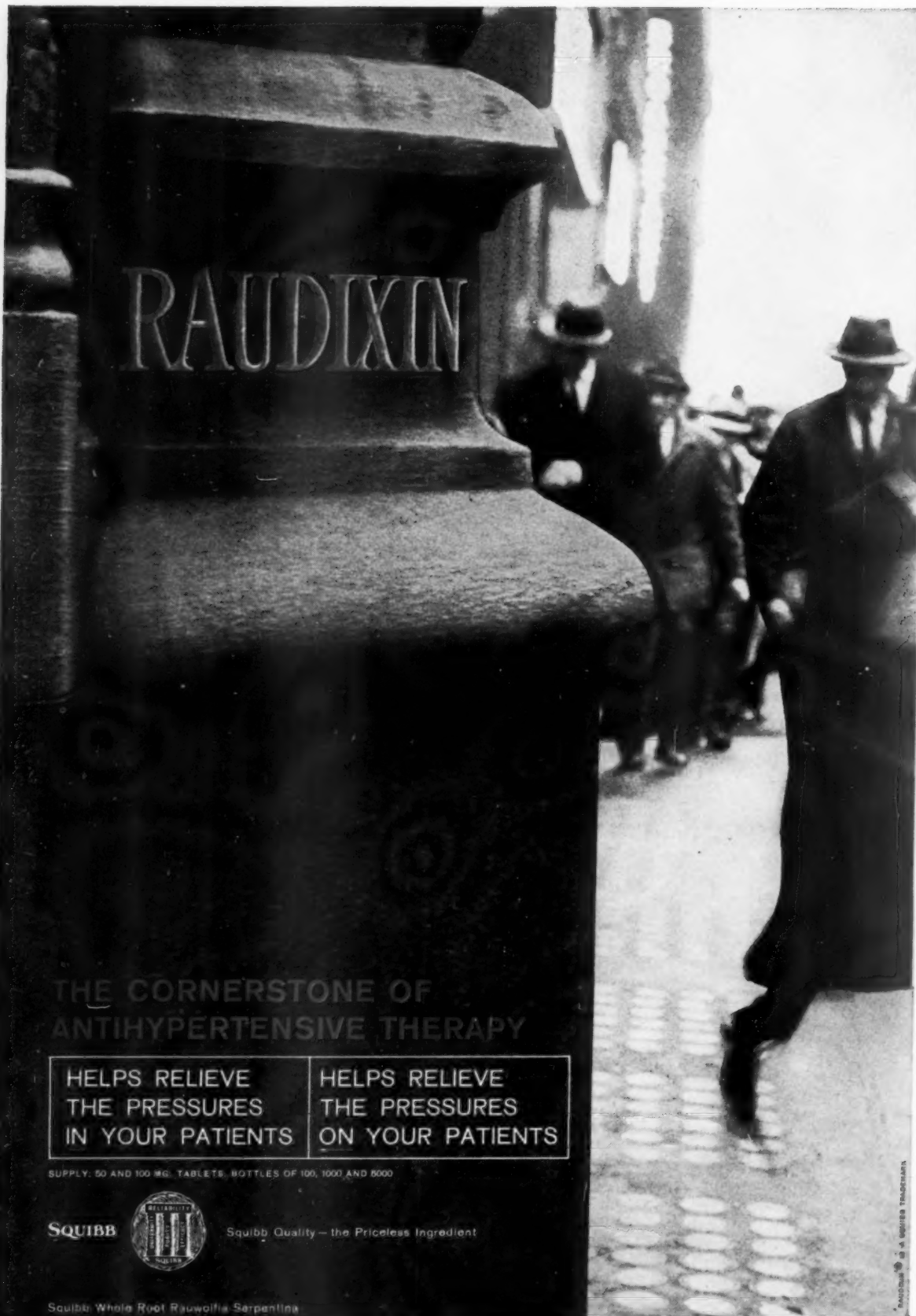
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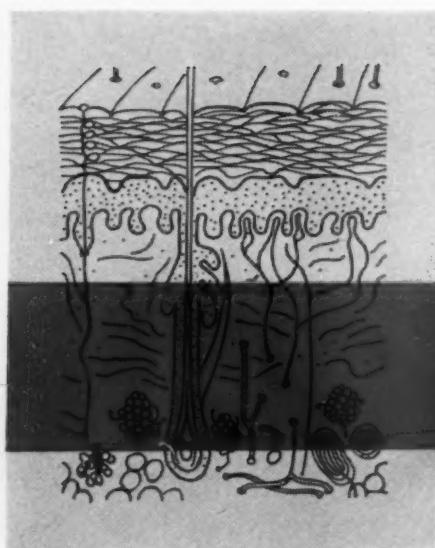
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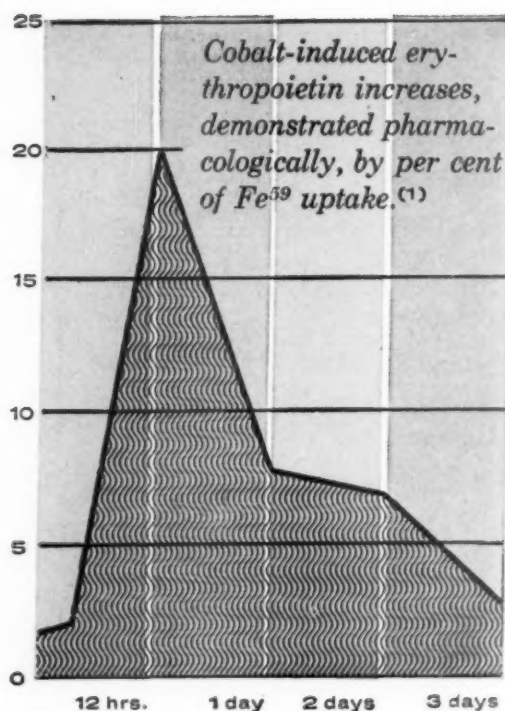
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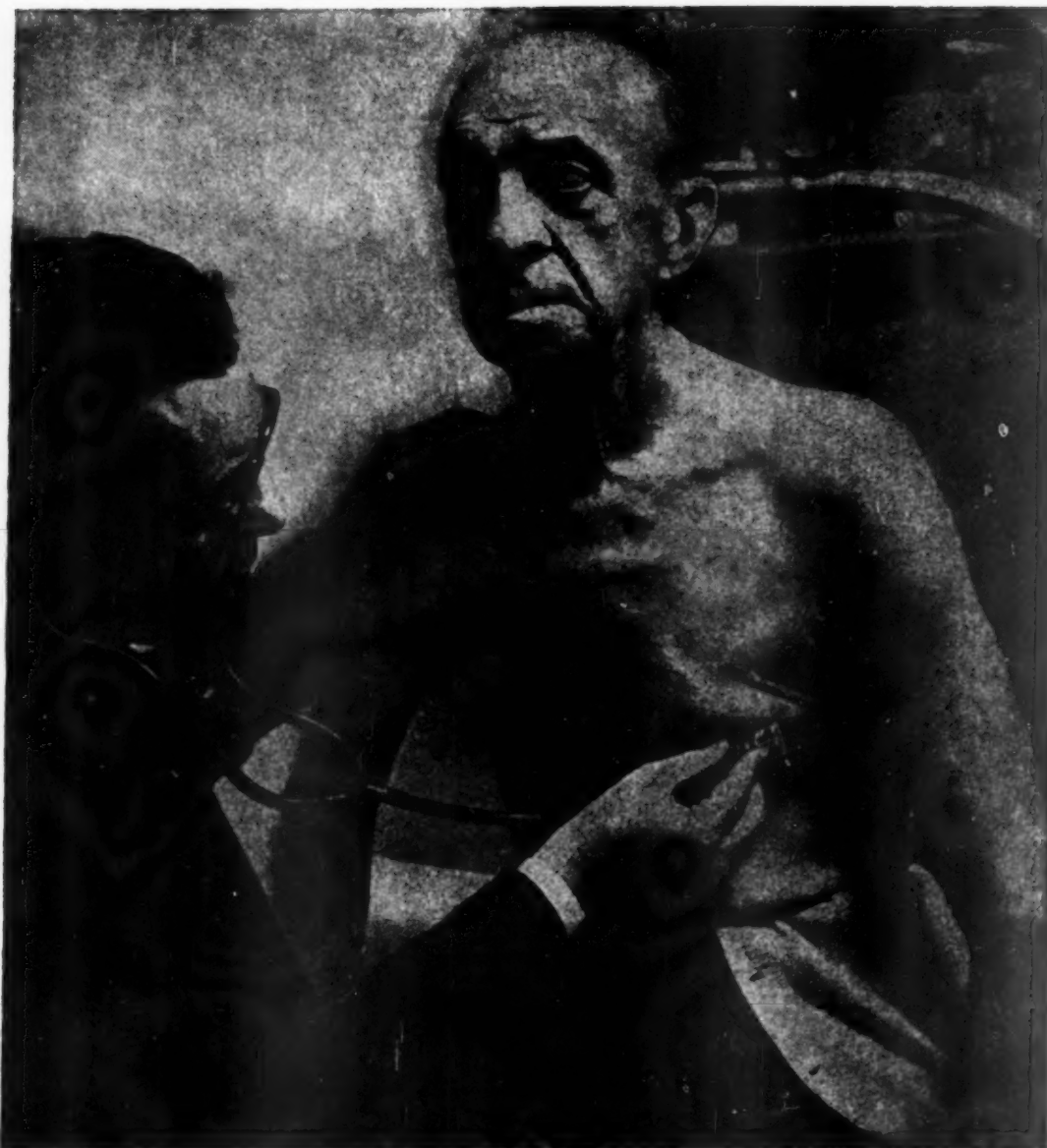
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